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Nebraska Prostate Cancer Research Program

PRINCIPAL INVESTIGATOR:
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CONTRACTING ORGANIZATION:
University of Nebraska
Omaha, NE 69198-6810

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14. ABSTRACT
Prostate cancer (PCa) is one of the major cancers threatening US males' life and has the highest incidence in American African males. For improving PCa treatments, better understanding the basic mechanism of this cancer is needed, which would depend on the training of more PCa researchers. The **purpose** of this grant is to train HBCU undergraduates with science major in PCa research. The **scope** of this training grant: Dr. Ming-Fong Lin of the University of Nebraska Medical Center (UNMC) and Dr. Shafiq Khan of Clark Atlanta University (CAU) have jointly instituted this training grant between UNMC and CAU for training CAU students in PCa research. The **major finding** and **up-to-date** report: Drs. Lin and Khan had identified interested CAU undergraduates and instituted a summer PCa research training program at UNMC where Dr. Lin recruited PCa scientist as mentors and CAU students learned translational research in labs. Students had spent the majority of their time working at bench on a research project. They also participated in seminar series that were specifically organized for summer undergraduate students and introduced them to broad areas of scientific investigation and advanced technological tools used in scientific discovery. During the academic year, students continued their training at CAU, which is arranged by Dr. Khan. This continuous training prepares students for a graduate career in biomedical sciences or medical school.

15. SUBJECT TERMS
Prostate Cancer, Training Grant, HBCU students, Biomedical Sciences, Graduate school, Medical school

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Annual Summary

1. **INTRODUCTION:**

The subject of this training grant is to train potential prostate cancer (PCa) researchers via a collaborative effort between University of Nebraska Medical Center (UNMC) at Omaha, Nebraska, and Clark Atlanta University (CAU) at Atlanta, Georgia. The conception of this Program is based on the interactions and collaborations between Dr. Ming-Fong Lin, the PI and a faculty mentor at UNMC, and Dr. Shafiq Khan, the collaborating-PI and a faculty mentor at CAU, since March 2004 with their first joint peer-reviewed publication in 2008 (1). **The purpose** of this proposal is to train undergraduate HBCU students from CAU to gain hand-on experience in performing research on PCa, a high incidence cancer in Africa American males (2), in UNMC, a PCa research-intensive environment. **The scope** of training grant is to train undergraduate students from CAU, a traditional HBCU, to gain hand-on experience in PCa research at UNMC Nebraska Prostate Cancer Research Program (NPCRP). These students will receive training not only in the lab but also in the class room in the format of seminars and visiting biotechnology companies which will broaden their scientific scopes. **The goal** is to encourage and to prepare HBCU students for academic career, i.e., they will either enter graduate school or medical school with training in, and understand of, PCa research or enter medical school. This will increase the number of PCa researcher at both basic science and clinical science levels.

2. **KEYWORDS:**

CAU, Clark Atlanta University; CDMRP, Congressionally Directed Medical Research Programs; DOD, Department of Defense; HBCU, Historically Black Colleges and Universities; INBRE, Idea Networks of Biomedical Research Excellence; NPCRP, Nebraska Prostate Cancer Research Program; PCa, Prostate cancer; UNMC, University of Nebraska Medical Center; UNMC SURP; UNMC Summer Undergraduate Research Program;

3. **OVERALL PROJECT SUMMARY:**

This report serves as the annual report of the funding support from CDMRP between 09/15/2014 – 09/14/2015, the second year of continuous support. Under current award, we recruited 4 CAU undergraduate students in Spring 2014 for their training in Summer 2014. These 4 students had gained hand-on experience in PCa research and made significant accomplishments. This section is in direct alignment with respect to each task outlined in the approved SOW. There were no technical or unexpected difficulties encountered and/or any deviations from the original Statement of Work. Per Guidelines, our training and research accomplishments following each task outlined in the approved Statement of Work are listed as follows:

(**Task 1 - 4 for 2014 Annual Report**)

Task 1: Announcement of the Year 1 Research Program (months 1-3)

Done. (a) Per approved SOW, in January 2013, Drs. Khan and Odero-Marah at CAU and Drs. Lin and Chaney at UNMC started to up-date the flier announcing the opportunity of conducting PCa research at UNMC. The wording in the flier was finalized by the end of January, and Drs. Khan, Odero-Marah and also other PCa faculty members at CAU Cancer Center immediately announced the opportunity by distributing the flier and verbal

announcements in their classes and also campus-wide posters by Dr. Khan's office in Feb 2013.
(b) The announcements included the criteria of eligibility, the requirement of documents, and the due date of application.
(c) Drs. Khan and Odero-Marah and Dr. Khan's office Ms. Bakari, the office manager, prepared all the necessary paper works and answered to all questions related to this opportunity. The final due date was set as by March 31, 2013.

Task 2: Selection of Trainees (month 4-6)

Done. (a) Dr. Khan's staff members went through all application files to ensure all application documents were complete and in place.
(b) Drs. Chaney and Lin visited CAU on March 17, 2013, met with Drs. Khan and Odero-Marah and discussed for student recruitment processes, and also met with 4 eligible student candidates on March 19-20, 2013. Drs. Odero-Marah sent all applicants' information to Dr. Chaney in the early April of 2013, after the due date of application. This year, we had 12 students who filed their applications.
(c) With the inputs of Dr. Odero-Marah, Drs. Chaney and Lin discussed all the applicants' qualification. Drs. Chaney and Odero-Marah also discussed and matched the candidate's interest with the research lab.
(d) Four successful applicants were identified and notified by e-mails from Dr. Chaney and also contacted by a secretary at CAU Cancer Center. The students were given a due date for replying of their acceptance.
(e) Dr. Chaney also coordinated the Housing issue at UNMC, and Ms. Karen at Department of Biochemistry and Molecular Biology, UNMC, mailed the first batch of documents including Housing information to students for their attention. With the support of Dr. Turpen, the NPCRP students continuously interacted with the INBRE program for student training, including attending the seminars and site visiting to various research facilities and biotech companies. We also registered those students for the UNMC SURP program.
(f) All students arrived on Monday of May 27, 2013. Dr. Chaney met students at the Omaha Eppley airport and drove them to the dorm. Drs. Chaney and Lin welcome them and had dinner with all students.

Task 3: Summer Research (months 7-9)

Done. (a) All students started their orientation on Tuesday of May 28 and continued on Wednesday of May 29. The INBRE Program had a Welcome Barbeque reception in the evening of May 29 and CAU students were also invited. In the afternoon of May 30, students reported to the matched lab and began their summer research in PCa. Dr. Chaney attended students' Monday seminar and met with students at least weekly. Dr. Chaney also gave students rides for grocery shopping weekly or whenever there was a need.
(b) Whenever possibly, Dr. Lin visited students in the labs to make sure everything was going well. He also met with all students after four weeks of training in research labs to discuss any potential issues or suggestions for improvement.
(c) Drs. Lin and Chaney invited Dr. Khan visiting UNMC, meeting with CAU trainees and faculty mentors and discussing with their research progress. Dr. Khan visited UNMC on July 7, 2013 (**Appendix #1**). He met with CAU students and had a lunch-meeting together to learn their progresses. Drs. Lin and Chaney and also other mentors Drs. Batra, Cheng, Datta

and Mehta all attended the lunch-meeting. After the lunch, Dr. Khan had a private meeting with students discussing any potential problem during their stays. Subsequently, Dr. Khan met with Drs. Lin and Chaney for an executive meeting discussing students' issues. Overall, all CAU students were very happy, enjoyed their stays and had obtained the hand-on experience in their research projects. They were very pleased with the research environment without any complain or suggestion.

Dr. Khan gave a scientific presentation on his research projects and also a brief introduction of research environment at the CAU to the UNMC community (**Appendix #1**). His presentation was well received by audience, including faculty members, post-doctoral fellows and graduate students. Dr. Khan also had meetings with mentors and other faculty members with similar research interests in PCa to discuss the potential of collaborations (**Appendix #2**).

(d) In the last week of training, all students prepared their posters. Due to their departure on Saturday of August 3, they were unable to present their posters in the UNMC Undergraduate Summer Research Program in August 9. Nevertheless, they learned how to present their data by preparing scientific posters, and these posters would also allow them to give presentations in future meetings. All the posters are attached (**Appendices #3-6**). Prior to their departure, Drs. Lin and Chaney met with students and had a farewell lunch as Dr. Lin's guests prior to their return to CAU.

Task 4: Evaluation of the Program (months 10-12)

Done. (a) Prior to their departure, all CAU students met with Drs. Chaney and Lin and other faculty mentors for a final lunch-meeting. We discussed any problem that occurred during their stays and any suggestion that will improve the training in future. The students were also asked to prepare the anonymous evaluations. From these evaluation and comments, we understand the issues raised by the students. We will implement these suggestions and improve our processes for a better training program.

Dr. Chaney met with Dr. Turpen and discussed the results of NPCRP training program during their INBRE Retreat. We appreciated very much for the strong supports from Dr. Turpen and the INBRE program to NPCRP. With such a cooperating effort, the CAU students could expose to different technologies applicable toward research and career development and also meeting with student peers for social events. We expect the continuing interactions in the up-coming years. Drs. Chaney and Lin met and discussed the potential improvement for the Training Program per students' comments.

(b) Drs. Khan and Odero-Marah and Ms. Bakari met with all CAU students at CAU and discussed the evaluation and concerns at the beginning of Fall semester (**Appendix #7**). All CAU students in general were very impressed and happy with the opportunity of training at UNMC, Omaha, NE. There was no other issue raised. Dr Khan then phoned Dr. Lin regarding the conclusion of meeting with students' evaluation and expressed students' positive attitude toward research experience in NPCRP at UNMC.

(c) Drs. Lin and Chaney met and discussed with mentors for inputs of improving the training quality for the following year. They also appreciated mentors' efforts.

(d) Based on the students' evaluation and comments, we believe we have a very successful training program. The success of the training program is clearly shown that our NPCRP-CAU trainees were eagerly participating to various scientific meetings and gave posters (**Key Research Accomplishments, Reportable Outcomes and Appendices #8-14**). We are very

pleased with the success of our Training Program, a joint effort by our colleagues and stuff member.

(**Task 5 - 8 for 2015 Annual Report**)

Task 5: Announcement of the Year 2 Research Program (months 13-15)

Done. (a) Per approved SOW, The availability of research training in prostate cancer was announced by different means, including CAU posters, the web sites, e-mail to students and the student clubs, and also verbal announcements in the classes at CAU campus by Drs. Khan and Odero-Marah and other prostate cancer research faculty members at CAU Cancer Center and Dr. Khan's office in Jan 2014.

(b) The announcement included the criteria of eligibility, the requirement of documents, and the due date of application.

(c) Drs. Khan, Odero-Marah and Dr. Khan's office Ms. Bakari, the office manager, answered to any question by potential student applicants and accept applications.

Task 6: Selection of Trainees (months 16-18)

Done. (a). Dr. Khan's staff members went through all application files to ensure all application documents were complete and in place.

(b). Drs. Lin and Chaney went to CAU, met with Drs. Khan and Odero-Marah, discussed application files and interviewed eligible student candidates.

(c). Drs. Lin, Chaney, Khan and Odero-Marah discussed and matched the candidate's interest with the research lab. The **match list** for UNMC Faculty with CAU students was as follows: Parmender Mehta - Quentin Loyd; Surinder Batra - Sierra Lawhorne; Ming-Fong Lin - Tashika Robinson; Pi-Wan Cheng - Brittney Carr.

(d). The successful applicants were notified by e-mails and regular mails and posted the list on board in CAU Prostate Cancer Research Center. The students were given a due date for replying of their acceptance.

(e). The Housing was coordinated by Dr. Chaney and the BMB Office Personnel at UNMC.

(f). Drs. Chaney met students at the Omaha Eppley airport and drove them to the dorm. Drs. Chaney and Lin had a welcome dinner with all students.

Task 7: Summer Research (month 19-21)

Done. (a). Dr. Chaney attended students' seminar weekly and met with students at least weekly (Appendix #19).

(b). Dr. Lin met with students after four-week of training in research labs.

(c). Drs. Khan and Odero-Marah visited UNMC, met with CAU trainees and discussed with their research progress. Dr. Odero-Marah gave a scientific presentation to BMB department including faculty members, post-doctoral fellows and graduate students (**Appendix #20**). It was well received.

(d). All CAU students gave scientific presentations in the UNMC Summer Undergraduate Research Program poster section (Appendix #21). Drs. Lin and Chaney met with students prior to their return to CAU.

Task 8: Evaluation of the Program (month 22-24)

Done. (a). Prior to students' departure, all CAU students met with Drs. Lin and Chaney and other faculty members during a lunch meeting. We discussed any problem that might occur

during their stays and any suggestions that might improve the training in future. The students were also asked to prepare anonymous evaluations which were collected by Dr. Chaney prior to their departure at airport (Appendix #22).
(b). Drs. Khan and Odero-Marah and also Ms. Bakari also met with students at CAU and discussed the evaluation and concerns at the beginning of Fall semester. Students' evaluations were further reviewed and discussed by Drs. Lin, Chaney, Khan and Odero-Marah.
(c). Dr. Chaney met with Dr. Turpen to discuss the results of NPCRP training program during their INBRE meeting. Drs. Lin and Chaney met with mentors and discussed students' evaluation for mentors' inputs to improve the training quality for the future training experience and to appreciate their efforts.
(d). Prepare the Annual report.

4. **KEY RESEARCH ACCOMPLISHMENTS:**
(**For 2014 Annual Report**)

- All CAU students attended the Monday seminar offered by the INBRE program through the entire period.
- All CAU students visited different research labs and biopharmaceutic companies offered by the INBRE program through the entire period.
- All CAU students attended Tuesday noon seminar offered by the UNMC Summer Undergraduate Research Program through the entire period.
- These students also visited regional universities and local BioPharm companies to expand their knowledge and scopes in future career developments.
- The UNMC INBRE IN ROADS, an INBRE communication, introduced the CAU students and reported the NPCRP efforts and the interactions between two programs.
- All CAU students prepared their results in posters.
- Our current CAU trainee Ms. Marisha Morris gave a poster presentation in the The Annual Biomedical Research Conference for Minority Students Annual National Symposium, November 15, 2013.
- Our current and former CAU trainees gave poster presentations in the 10^{th} Annual National Symposium on Prostate Cancer March, 16-19, 2014.

(**For 2015 Annual Report**)

- All CAU students attended the UNMC Summer Undergraduate Research Program seminar through the entire period (Appendix #19).
- All CAU students prepared their results in posters.
- All CAU students gave presentations in UNMC Summer Undergraduate Research Program by the end of their training (Appendixes #21, 23-26).
- Our current CAU trainee Ms. Tashika Robinson was awarded by the AAMC Fee Assistance Program.

- Our former trainee Ms. Lynette Leffall was admitted for the Class 2016 at the Ohio University Heritage College of Osteopathic Medicine,
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5. **CONCLUSION:**

The purpose of this award is to train HBCU undergraduate students from CAU to gain hand-on experience in performing PCa research in a research-intensive focus group, the UNMC Nebraska Prostate Cancer Research Program (NPCRP). We are very pleased with the outcomes for the continuing success of the training program at UNMC. These students have received training not only in the lab but also in the class room in the format of seminars and visiting biotechnology companies. Our goals of training are to encourage and to prepare HBCU undergraduate students for academic career in graduate school or medical school with training in, and understand of, prostate cancer research. We propose that by this way, we can increase the number of PCa researcher from the minority group at both the basic science and the clinical science levels. With this award support from the DOD PCa Research Program, as evidenced by the scientific outcomes of student posters and student comments, we are very excited by the success of our training program. It is clearly evidenced by current and former students who are eagerly participating various scientific meetings and gave posters as described in accomplishments in sections: **Key Research Accomplishments, Publication, Abstracts and Presentations, and Reportable Outcomes (**Appendices #19). The results of research have been put together and included for preparing a solid, peer-reviewed scientific article (manuscript in preparation). We are expecting that more exciting results will be done in the up-coming years.

6. **PULICATIONS, ABSTRACTS, AND PRESENTATIONS:**

(All NPCRP Trainees including current and former are identified in **bold**.)
(**For 2014 Annual Report**)

PULICATION:

a. Muniyan, S., Chou, Y.W., Ingersoll, M.A., **Devine, A.**, **Morris, M.**, Odero-Marah, V.A., Khan, S.A., Chaney, W.G., Bu, X.R., Lin, M.F. (2014). Antiproliferative activity of novel imidazopyridine derivatives on castration-resistant human prostate cancer cells. Cancer Letters 353: 59-67. (PMID: 25050738) (NIH MS615152, Publ.ID: CAN11924)

ABSTRACTS AND PRESENTATIONS:

a. Vo, B.T., Boseman, M., **Leath, C.**, Battle, S., Khan, S.A. (2014). Intracellular mechanisms of arcadia abolished TGF-beta induced proteasome degradation of Ski protein in prostate cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p.62.
b. Chunduri, H., Vo, B.T., **Leath, C.**, Khan, S.A. (2014) Gai is critical for TGF-beta1, PGE2, OXT and EGF induced migration in prostate cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p.66.
c. Ghotbaddini, M., Tran, C., Richmond, O., **Aaron, LaTayia**, Powell, J.B. (2014). Inhibition of constitutive aryl hydrocarbon receptor (AHR) signaling attenuates androgen

independent signaling and growth in C4-2 prostate cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, mp.70.

d. **Leath, C.**, Jiang, C., Davis, J. Khan, S. (2014). Inhibiting RAC 1-GTPase activity in ovarian cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p.83.
e. **Loyd, Q.**, Smith, B.N., Henderson, V., McKeithen, D., **Morris, M.**, Nagappan, P., and Odero-Marah, V.A., (2014) Snail transcription factor regulates cathepsin L and maspin in prostate cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p.84.
f. **Morris, M.**, Muniyan, S., Dwyer, J.G., Bu, X., Lin, M.F. (2014). Novel imidazopyridine derivatives inhibit androgen-independent prostate cancer cell proliferation. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p. 87.
g. **Wells, J.**, Petrosyan, A., Cheng, P.W. (2014) Non-muscle myosin IIA-mediated golgi fragmentation in advanced prostate cancer cells. The 10th Annual National Symposium on Prostate Cancer March, 16-19, 2014, p.98.

PRESENTATION:

a. Marisha Morris, Sakthivel Muniyan, Jennifer G. Dwyer, Xiu Bu, Ming-Fong Lin (2013). Novel imidazopyridine derivatives inhibit androgen-independent PCa cell proliferation. The Annual Biomedical Research Conference for Minority Students Annual National Symposium, November 15, 2013.

(**For 2015 Annual Report**)

PULICATION:

a. Ingersoll, M.A., Lyons, A.S., Muniyan, S., D'Cunha, N., **Robinson, T.**, Hoelting, K., Dwyer, J.G., Bu, X.R., **Batra, S.K.**, **Lin, M.F.** (2015). Novel Imidazopyridine Derivatives Possess Anti-tumor Effect on Human Castration-Resistant Prostate Cancer Cells. PLoS ONE 10(6):e0131811. doi: 10.1371/journal.pone.0131811. eCollection 2015. (PMID: 26121643); PMCID: PMC4487901. **(**Appendices #27).

ABSTRACTS AND PRESENTATIONS:

a. **Britney Carr**, Armen Petrosyan, Ganapati Bhat and Pi-Wan Cheng. Alcohol Induction of Golgi Fragmentation and Prevention of Golgi Targeting of Selective Glycosyltransferases in Androgen Sensitive Prostate Cancer Cells. UNMC Summer Undergraduate Research Program Poster. August 7, 2014. **(**Appendices #23).
b. **Quentin Loyd**, Ray, A., Katoch, P., Kelysey, L., and **Parmender Mehta**. Regulation of gap junction protein in prostate cancer cells. UNMC Summer Undergraduate Research Program Poster. August 7, 2014. **(**Appendices #24).
c. **Lawhorne, S.L**., Muniyan, S., Seshacharyulu, P., **Lin, M.F.**, **Batra, S.K.** (2014). Inhibition of SHH signaling enhances Docetaxel efficacy in castration-resistant prostate cancer cells. UNMC Summer Undergraduate Research Program Poster. August 7, 2014. **(**Appendices #25).
d. **Robinson, T**., Ingresoll, M., Muniyan, S., Morris, M., Hoelting, K., Bu, X.R., Batra, S.K., **Lin, M.F.** (2014). Inhibition of Tumorigenicity of Castration-Resistant Human

Prostate Cancer Cells by Imidazopyridine Derivatives. UNMC Summer Undergraduate Research Program Poster. August 7, 2014. **(**Appendices #26).

7. **INVENTIONS, PATENTS AND LICENSES:**
None.

8. **REPORTABLE OUTCOMES:**
(**For 2014 Annual Report**)
- All our CAU trainees had gained valuable hand-on experience in PCa research.
- They learned translational research in PCa, including biomarker discovery, molecular pathogenesis, signaling transduction and experimental therapy.
- CAU student Ms. Marisha Morris gave poster presentations in the The Annual Biomedical Research Conference for Minority Students Annual National Symposium, November 15, 2013.
- With inputs of CAU trainees, our research results have identified a novel group of compounds that may be used in advanced PCa therapy.
- Our results have been published as a peer-reviewed article for potential PCa therapy. (Publication #1: Muniyan, S., et.al. (2014). Antiproliferative activity of novel imidazopyridine derivatives on castration-resistant human prostate cancer cells. Cancer Letters 353: 59-67.)
- Our current and former CAU trainees gave poster presentations in the 10^{th} Annual National Symposium on Prostate Cancer March, 16-19, 2014.
- In the 10^{th} Annual National Symposium on Prostate Cancer March, 16-19, 2014, a total of 7 posters were contributed by our CAU trainees.
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(**For 2015 Annual Report**)
- All our CAU trainees had gained valuable hand-on experience in PCa research.
- They learned translational research in PCa, including biomarker discovery, molecular pathogenesis, signaling transduction and experimental therapy.
- With inputs of CAU trainees, our research results have identified a novel group of compounds that may be used in advanced PCa therapy.
- Our results have been published as a peer-reviewed article for potential PCa therapy. (Publication a, Appendix #27.)
- Our former trainee Ms. Lynette Leffall was admitted for the Class 2016 at the Ohio University Heritage College of Osteopathic Medicine.
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9. **OTHER ACHIEVEMENTS:**
None. There is no other achievement.

10. **REFERENCES:**

1. Dillard, P.R., **Lin, M.F.**, and **Khan, S.A.** (2008). Androgen-independent prostate cancer cells acquire the complete steroidogenic potential of synthesizing testosterone from cholesterol. Mol. Cellu. Endo. 295:115-120.
2. Siegel, R., Naishadham, D., and Jemal, A. (2012). Cancer Statistics, 2012. CA Cancer J Clin 62: 10-29.

11. **APPENDICES:**

(**For 2014 Annual Report**)

1. Appendix #1: Announcement by BMB for Dr. Shafiq Khan's scientific presentations at UNMC.
2. Appendix #2: Itinerary for Dr. Shafiq Khan meeting with NPCRP faculty members for discussing future collaborations.
3. Appendix #3: Poster prepared by CAU trainee Chelesie Leath
4. Appendix #4: Poster prepared by CAU trainee Jaclyn Welles
5. Appendix #5: Poster prepared by CAU trainee Tashika Robinson
6. Appendix #6: Poster prepared by CAU trainee Mohammed Ghislain Djibo.
7. Appendix #7: CAU student evaluations.
8. Appendix #8 (p.62): Poster presentations by Ms. Chelesie Leath in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
9. Appendix #9 (p.66): Poster presentations by Ms. Chelesie Leath in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
10. Appendix #10 (p.70): Poster presentations by Ms. LaTayia Aaron, our 2011 trainee, in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
11. Appendix #11 (p.83): Poster presentations by Ms. Chelesie Leath in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
12. Appendix #12 (p.84): Poster presentations by Quentin Loyd in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
13. Appendix #13 (p.87): Poster presentations by Ms. Marisha Morris, our trainee, in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
14. Appendix #14 (p.98): Poster presentations by Ms. Jaclyn Wellws in the 10th Annual National Symposium on Prostate Cancer by CCRTD, CAU, March 16-19, 2014.
15. Appendix #15: Peer-reviewed scientific publication with inputs from CAU trainees (Cancer Letters, 2014).

(**For 2015 Annual Report**)

a. Appendix #19: UNMC Summer Undergraduate Student Research Program Handbook.
b. Appendix #20: CAU faculty member visted UNMC and gave a presentation.
c. Appendix #21: UNMC SURP Research Colloquium, August 2014.
d. Appendix #22: NPCRP Summer Student Evaluation, August 2014.
e. Appendix #23: Poster presentation by Ms. Britney Carr at UNMC Summer Undergraduate Research Program Poster. August 7, 2014.
f. Appendix #24: Poster presentation by Mr. Quentin Loyd **at** UNMC Summer Undergraduate Research Program Poster. August 7, 2014.

g. Appendix #25: Poster presentation by Ms. **Lawhorne, S.L**. at UNMC Summer Undergraduate Research Program Poster. August 7, 2014.
h. Appendix #26: Poster presentation by Ms. **Robinson, T**. at UNMC Summer Undergraduate Research Program Poster. August 7, 2014.
i. Appendix #27: Ingersoll, M.A., Lyons, A.S., Muniyan, S., D'Cunha, N., **Robinson, T.**, Hoelting, K., Dwyer, J.G., Bu, X.R., **Batra, S.K.**, **Lin, M.F.** (2015). Novel Imidazopyridine Derivatives Possess Anti-tumor Effect on Human Castration-Resistant Prostate Cancer Cells. PLoS ONE 10(6):e0131811. doi: 10.1371/journal.pone.0131811. eCollection 2015. (PMID: 26121643); PMCID: PMC4487901.

12. TRAINING AWARD & ACTIVITIES:

List of Training Activities include seminar and visiting.

(**For 2014 Annual Report**)

1. Appendix #16: An example of INBRE schedule including seminar and visiting.
2. Appendix #17: An example of Summer Undergraduate Student Program including seminar and visiting.
3. Appendix #18: A list of NPCRP mentors and their research expertise.

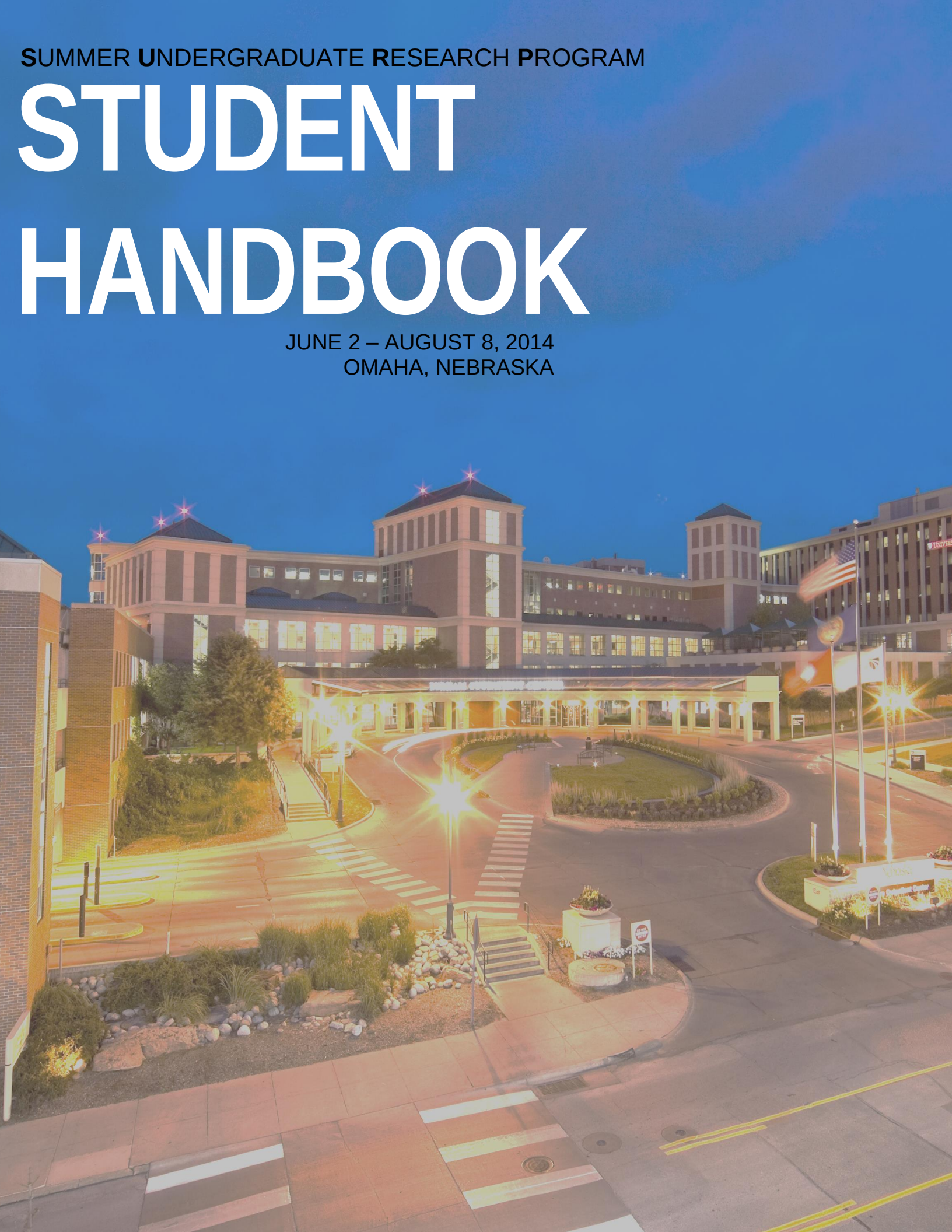
(**For 2015 Annual Report**)

4. Appendix #19: An example of 2014 Summer Undergraduate Student Program including seminar and visiting.
5. Our CAU trainee Ms. Tashika Robinson was awarded by the AAMC Fee Assistance Program.
6.

SUMMER **U**NDERGRADUATE **R**ESEARCH **P**ROGRAM

STUDENT HANDBOOK

JUNE 2 – AUGUST 8, 2014
OMAHA, NEBRASKA



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Jeffrey Gold, MD Chancellor

H. Dele Davies,
PhD Vice Chancellor for
Academic Affairs Dean for
Graduate Studies

Jennifer Larsen, M.D.
Vice Chancellor for Research

Courtney Fletcher, Pharm.D.
Dean for College of Pharmacy

Bradley E, Britigan, M.D.
Dean for College of
Medicine

UNMC | UNIVERSITY OF NEBRASKA MEDICAL CENTER

Dear SURP Student,

We would like to extend a warm UNMC welcome to all of this year's participants in the Summer Undergraduate Research Program (SURP). SURP at UNMC brings together an extraordinary group of distinguished faculty, clinicians, researchers, and staff to provide a unique ten-week experience for you. Our diverse team of faculty and staff will explore areas of research, provide mentoring, and make sure you get the individual attention necessary for you to excel.

You will have the opportunity for:

- Hands-on laboratory experience with a research team
- Engagement in weekly seminars with topical areas such as health policy and research careers
- Present your own research at a Poster Symposium
- Daily interactions with research faculty, staff, and students
- Friendships that will last a lifetime

We wish you the best as you make final preparation for your arrival in Omaha to participate in SURP 2014.

WHO WE ARE

A vital enterprise in the nation's heartland, the University of Nebraska Medical Center has its eye on improving the future of health care in Nebraska and beyond.

As Nebraska's only public academic health sciences center, UNMC is committed to the education of a 21st century healthcare workforce, to finding cures and treatments for devastating diseases, to providing the best care for patients, and to serving our state and its communities through award-winning outreach. UNMC is committed to embracing the richness of diversity, and is a major economic engine for the state of Nebraska.

Led by Chancellor Jeffrey Gold, M.D., UNMC has six colleges and two institutes, serving more than 3,100 students in more than two dozen programs. In 2009, U.S. News & World Report ranked UNMC's rural health medicine program 15th and its primary care program 17th. The university's physician assistant, physical therapy, pharmacy, and nursing programs also were ranked as among the top in the country.

COLLEGE OF PHARMACY

The Mission of the College of Pharmacy at the University of Nebraska Medical Center is to improve the health of Nebraska through premier educational programs for pharmacists, other health professionals and graduate and post-graduate students, conduct innovative research and provide highest quality patient care and outreach to underserved populations.

EPPLEY: INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES

The mission of the Eppley Institute for Research in Cancer and Allied Diseases is to develop superior research programs that will provide a better understanding of the causes of cancer, improve the methods for diagnosis of cancer and improve the methods for the treatment and prevention of cancer and similar disorders. They will also provide outstanding graduate and post-graduate educational opportunities in cancer research.

COLLEGE OF MEDICINE

The mission of the University of Nebraska Medical Center College of Medicine is to improve the health of Nebraskans through premier education programs, innovative research, quality patient care and outreach to underserved populations. We strive to be a regional and national leader in the education of primary care health professionals, the application of information technology and other major areas of healthcare need.

UNIVERSITY OF NEBRASKA MEDICAL CENTER

MISSION

The mission of the University of Nebraska Medical Center is to improve the health of Nebraska through premier educational programs, innovative research, the highest quality patient care, and outreach to underserved populations.

OMAHA

Omaha is an ethnically and racially diverse community, and with a population of over 700,000 inclusive, is Nebraska's largest city. While the Henry Doorly Zoo has long been of of Nebraska's top tourist attractions, Omaha boasts many other outstanding attractions, including a drive-through Wildlife Safari Park; the Strategic Air and Space Museum, a Smithsonian Institute affiliate devoted to U/S. military aircraft, missiles, and science; Girls and Boys Town, funded by Father Edward Flanagan; and, of course, the NCAA College World Series.

Omaha is a true cultural center with over 20 museums and fine arts center, including the Joslyn Art Museum (a Smithsonian Institute affiliate), Durham Western Heritage Museum, El Museo Latino, The Rose Theatre, and the Holland Performing Arts Center. Each summer, Omaha is host to number concert and theatre series, including Jazz on the Green, Omaha Riverfront Jazz and Blues Festival, and Shakespeare on the Green. The CentryLink Center is a top venue for touring musicians, national rodeos, and sporting events. The historical Old Market area and recently developed 'NoDo' in downtown Omaha provide many cultural/artistic shopping and dining opportunities. MidTown Crossing, very close to UNMC campus, and Aksarben Village, are two developing entertainment and shopping hubs.

University of Nebraska Medical Center

www.unmc.edu | www.unmc.edu/sorrellcenter/index.htm

SURP at UNMC

402.559.3809

RSE@unmc.edu | www.unmc.edu/RSE

CLARKSON COLLEGE

http://www.clarksoncollege.edu/studentlife/housing/

Omaha

www.visitomaha.com | www.accessomaha.com | www.omaha.com

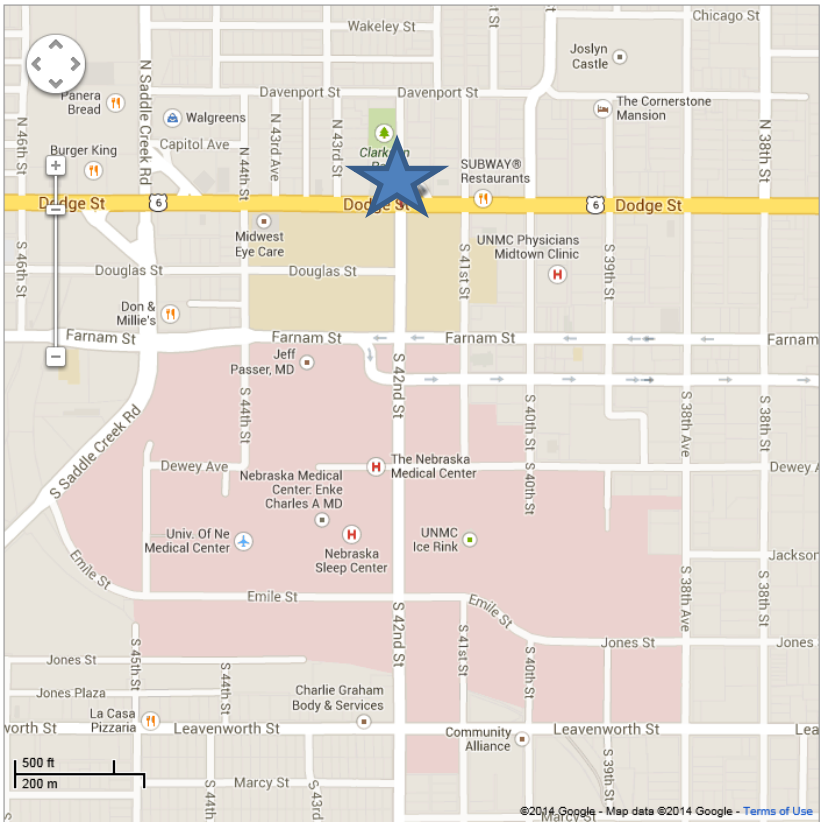
ARRIVAL

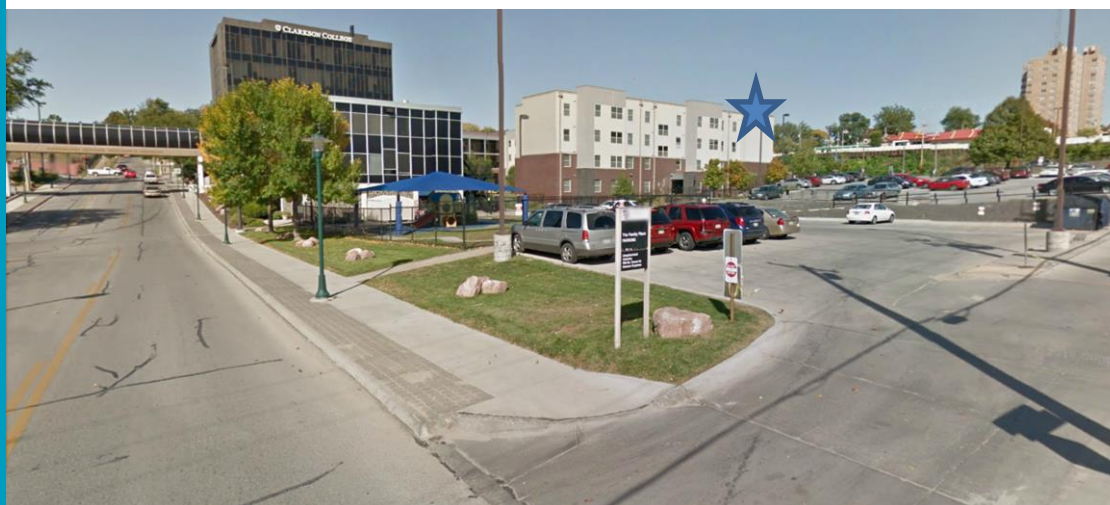
CHECK-IN

If you are staying at Clarkson College, upon arrival in Omaha, you will check in at the residence hall, located at 104 S. 42 Street #1 Omaha, NE 68131. If you are arriving by car, please use this map to direct you to the residence hall or use mapquest.com to give you directions.

When you check in, you will confirm your housing accommodations; receive your room assignment, a key to your apartment, room, and a mailbox for your use during your stay.

= Clarkson College





WHAT TO BRING

Alarm clock

Calling card or cell phone + charger

Bank card (deposits and cash)

Toiletries (soap, shampoo, deodorant, etc.)

Shower shoes- flip flops

Shower caddy

Robe/ pajamas/ lounge wear

Notebook paper, pens, pencils, etc.

Clinical ward attire (slacks/skirts, shirts/blouses)

Reception attire (tie, dress/slacks, skirt, suit/jacket)

Rainwear and umbrella

Comfortable business casual clothes

Tennis shoes/ comfortable walking shoes

Flashlight with batteries

Light jacket or sweater

Light blanket (concert, plays, and other events in area parks)

Medications

NEBRASKA WEATHER

Nebraska's summer weather can be unpredictable. The summers are hot and humid, but the weather may vary during the duration of the program. The residence hall is air conditioned, and it is recommended that you bring a blanket for the cooler nights. It is also recommended that you bring a light jacket or sweater for the lecture hall and classrooms. Additionally, Nebraska often has rain and severe thunderstorms. Please bring rain gear - jacket/umbrella, as well as a flashlight for your dormitory room.

STUDENT HEALTH SERVICES

SURP students are required to provide their own health insurance. SURP students are not considered UNMC students through Student Health. If you would like to be seen at the clinic, you'll need to go in as a regular patient. If no appointments are available for the day, you'll be referred to the ER if necessary. The Student Clinic is located on the UNMC campus on Level 3 of the Durham Outpatient Center.

For more information, including prices visit their website at www.studentinsurance.com

PARKING

To park at UNMC you'll need to pay $15/month. Once you have your UNMC I.D. badge, you can visit Parking Services located in the Student Center, second floor in the Bookstore. Hours of operation: Monday – Thursday: 7:30AM – 4:30PM, Friday: 7:30AM – 4:00PM

STUDENT HEALTH CLINIC HOURS

Appointments	402.559.7200
Mon – Fri	8AM – 4:30PM
Sat (Urgent care only)	8AM – 12PM

Photo ID required for appointments
Your Pharmacy needs to fax prescription refill requests to: 402.559.5550

LIVING

HOUSING

Clarkson College will provide SURP students enjoy a convenient, comfortable, unique living and learning experience. The Student Village includes an apartment-style residence hall and courtyard. The residence hall is conveniently located right across from the main campus of University of Nebraska Medical Center.

To secure housing, please contact Clarkson College and tell them you are from UNMC's SURP program. It will be the responsibility of each student (unless otherwise stated by the department) to select their housing of choice, sign a contract, coordinate accommodations and facilitate payment. *Please note this is not an exhaustive list of all expenses or amenities.

The new facility for on-campus living includes:

- Controlled access
- 24-hour staff
- Laundry facility

Typical Floor Plan & Amenities:

- Four private, furnished bedrooms
- Furnished living room
- Two private bathrooms with double vanities
- Spacious, fully equipped kitchen
- Dining/study counter
- Cable hookups
- Internet availability in each bedroom

Contact:
Student Housing Office
Clarkson College
104 S. 42 Street #1
Omaha, NE 68131
PH: 402-991-4825 TF: 800-647-5500
housing@clarksoncollege.edu

For more information visit: http://www.clarksoncollege.edu/studentlife/housing

MIDTOWN CROSSING

"It's bold but sophisticated. World class but Omaha proud. It's energy and community, the place where fresh meets the future. Always on. Always inviting. Always in style**.** It's Friday night with friends at an exclusive, four-star restaurant and a Saturday morning stroll through beautiful Turner Park. It's luxury living with all the amenities. Convenience. Not Compromise. It's time you experienced 222,000-square-feet of diverse retail and extraordinary diversion**.** An unprecedented development in the middle of it all. Mid Summer to Mid Winter. Mid Morning to Mid Night. And everything in between."

SHOPPING

A Wohlner's Grocery, Walgreens and many fast food and restaurants are located within easy walking distance of Clarkson College.

EVENTS

During the ten weeks in Omaha, scholars will have the opportunity to explore the Omaha community. Students can check out Omaha's shopping, movies, dances, concerts, and other special events such as Shakespeare on the Green, Taste of Omaha, and the Omaha Arts Festival.

For the sports and recreation enthusiast, Omaha is a great place to be. Annually, it plays host to the NCAA Men's College World Series and the Cox Classic Golf Tournament, a PGA Nationwide tour even. We are a rotating host for NCAA Women's Volleyball Championship and the US. Olympic Swim Trials. We have NCAA College Hockey, Basketball and AAA Baseball – The Omaha Storm Chasers. Omaha has numbers parks, 14 golf courses, 15 public swimming pools, miles of biking paths, ice skating rinks, inner-city lakes for fishing, picnicking, and more!

Check out the exciting events occurring in Omaha during your stay:

- Summer Arts Festival (June 6-8)
- Vibes at Village Pointe (outdoor concert series)
- Nebraska Wind Symphony
- Omaha Riverfront Jazz & Blues Festival
- Race, Omaha Community Playhouse (May-June)
- Rose Theatre
- NCAA Men's College World Series (June 13)
- Omaha Storm Chasers (Triple A affiliate of the Kansas City Royals)
- El Museo Latino
- Joslyn Art Museum
- NE Jewish Historical Society & Riekes Museum
- The Western Heritage Durham Museum
- Bank of the West Celebrates America Concert
- Hot Shops Art Center
- FunPlex (water & amusement park)
- Mahoney State Park
- Strategic Air & Space Museum
- Old Market dining and shopping
- Santa Lucia Festival
- Bemis Center for Contemporary Arts

BANKING

Metro Credit Union, adjacent to UNMC, provides free check-cashing services to SURP. Other banks near the UNMC and Clarkson Campuses are:

- American National Bank
- Bank of America
- Bank of the West
- First National Bank
- Security National Bank
- US Bank
- Wells Fargo Financial Bank

FAITH OPPORTUNITIES

There are a several places of worship within walking distance of Clarkson College. Let us know if you are interested and we will connect you!

PROFESSIONALS

Event	Date	Time	Location	Speaker
Orientation	Monday, June 2nd	9:30AM – 11:30AM	Durham Research Center1 1002	Varies
Compliance Training	Monday, June 2nd	12:30PM – 3:15PM	Durham Research Center1 1002	Sara Ward
Tuesday Lunch Seminar	Tuesday, June 10th	12:00PM – 1:00PM	Eppley Science Hall 3010	Dr. Romberger
Tuesday Lunch Seminar	Tuesday, June 17th	12:00PM – 1:00PM	Eppley Science Hall 3010	Adam Beck, Kaplan
Tuesday Lunch Seminar	Tuesday, June 24th	12:00PM – 1:00PM	Eppley Science Hall 3010	Dr. Solheim Cancer Research
Tuesday Lunch Seminar	Tuesday, July 1st	12:00PM – 1:00PM	Eppley Science Hall 3010	Dr. Joyce Black Legal Expert Witness
Tuesday Lunch Seminar	Tuesday, July 8th	12:00PM – 1:00PM	Eppley Science Hall 3010	Dr. Singh
Tuesday Lunch Seminar	Tuesday, July 15th	12:00PM – 1:00PM	Eppley Science Hall 3010	Dr. Gendalman
Tuesday Lunch Seminar	Tuesday, July 22nd	12:00PM – 1:00PM	Eppley Science Hall 3010	Dr. Schultz
Tuesday Lunch Seminar	Tuesday, July 29th	12:00PM – 1:00PM	Eppley Science Hall 3010	Dr. Wang
Poster Symposium	Thursday, August 7th	10:00AM - 12:30PM	TBA	N/A
Closing Banquet	Thursday, August 7th	1:00PM	Storz Pavillion	N/A

GRADUATES

Meet SURP Graduates: Young Kim, Leo Tyler & Jamie Holland

What sparked your interest in SURP?

Young Kim: "My interest in SURP and research within the sciences stem from my belief that "truth has nothing to fear from investigation. As a Christian, I believe that research and scientific inquiry is a great endeavor: "It is the glory of God to conceal a matter; to search out a matter is the glory of kings" (Proverbs 25:2). By participating in SURP and research, I was afforded the opportunity to learn a small portion of the fantastic and intricate function of the human body."

Leo Tyler: "I felt the program would contribute heavily not only to my growth academically but also personally. The idea of being on the campus that I hoped to someday be studying as a medical student and possibly working as a physician was exciting. I also felt that the experience would give me an opportunity to connect with people within my desired profession and allow me to gain more insight into the world of medicine, healthcare and academia."

Jamie Holland: "The ability to participate in cutting edge research and the opportunity to work with a professional and knowledgeable staff sparked my interest in SURP."

What is it like to be a college student in a distinguished investigator's laboratory?

Kim: "Prior to meeting my PI, I was a little intimidated by the fact that my PI was a professor at the med school. However, my PI was extremely warm, friendly, and inviting, and he helped to get me acclimated to the lab environment. Overall, I had an absolutely enriching research experience working in his lab, and I came out of UNMC SURP appreciating the fact that I was privileged to work in such a lab environment."

Tyler: "My experience was different from most of the other students with which I connected with, in that I was not in a laboratory at all. My everyday experience took place in my preceptor's office (Dr. Jack Turman) and at Omaha Home for Boys, where I was allowed to be a part of my groups' research project which dealt mostly with people, i.e. human subjects. But to answer what I believe the question is getting at…I was given a very warm welcome upon arrival and made to feel right at home. I was challenged and prompted to engage in our group discussions around our research project (the Connections Project), and assigned several research tasks from which I learned much. In hindsight I was given what seemed to be just the right amount of time and guidance to complete my given tasks without too much help and was well informed in how to complete the task. I feel this dynamic is key in an internship to promote the most growth/learning. Expose the student/intern to a challenge with enough information to complete the task but not so much help that they aren't challenged and allow them to learn through the experience. So in other words I was given a task, some bullet points on what was thought to be fundamental and the rest was left up to me to finish, which I feel gave me just the right amount of frustration and satisfaction that was key to a great learning experience."

Holland: "As a SURP student I had the opportunity to learn about research protocols, current research projects, and the up-to-date technology used by research scientists in state-of-the-art laboratories."

What was the highlight during your time here?

Kim: "Some highlights while participating in SURP include meeting people from across the country and having the opportunity to work on a fantastic project. I also had fun staying up late with some friends working on our respective posters (for the poster presentations). In terms of highlights within the lab, I was floored when I had the opportunity to experience immunofluorescence.

Tyler: "There were many highlights during the summer, but I think the most important and influential times of my internship experience were when I was able to converse with Dr. Turman. I learned much in regards to research methods, epidemiology in general, physical therapy, and just the life experiences of my colleagues. Much can be learned from people in a well-experienced position. To be in a lab researching is great, but other things can also be learned by just talking to people who have great experiences to share."

Holland: "Interacting with others while developing my research skills and exploring the many graduate programs available at UNMC were among the highlights of my SURP experience."

What was it like meeting the other undergraduate researchers?

Kim: "I had the opportunity to live in the Clarkson Apartment dorm with 3 other undergrad researchers from across the country. After coming back from our respective research departments, we were able to share what happened through our day and hang out together. In our leisure times and on weekends, we traveled around Nebraska, hung out at the College World Series, went shopping, explored the Omaha Zoo, and played basketball (it was always fun watching the NBA Finals together as we all rooted for different teams)."

Tyler: "I appreciated the times we were allowed to come together with our fellow undergraduate researchers. It was encouraging to see fellow students with a passion to pursue their career goals and hear their stories and future aspirations."

Holland: "I enjoyed the opportunity to interact with a diverse group of students from around the country who shared my interests in medical research. I welcomed the opportunity to exchange ideas and information with them. This experience not only enhanced my research skills, but my communication skills as well."

How will this experience stay with you going forward?

Kim: "The lab experience I took from SURP has helped me in my cellular biochemistry lecture and lab. Furthermore, I am sure that my lab experience will help me be a well-rounded individual as I apply to medical school this upcoming year!"

Tyler: "I will take the knowledge and wisdom that I learned from my experience, from both the research and interactions within my group."

Holland: "The SURP experience helped me develop background knowledge related to internal medicine that was applicable to my current studies. It also sparked my interest in continuing my education in a health care related field."

What advice do you have for this year's participants?

Kim: Come in ready to work and learn from both peers and mentors! You reap the rewards of what you sow -- if you want a fulfilling research experience, be prepared to make the most of your hours in the lab! Cultivate relationships with peers and make the most out of your summer!

Tyler: First of all be grateful and humble yourself for having been given the opportunity afforded to you. At no other time, aside from maybe graduate school, will you ever be able to be paid to simply learn without too many demands in return. Know however, that you will get out of your internship only what you put into it. In other words, life is what you make it; in this case your internship is what you make it. Be willing to always ask questions in order to get a better understanding of the bigger picture, because the whole internship process is really a very good knowledge, experience, and wisdom absorbing opportunity. Be like a sponge and soak up all that is around you during you visits. I would advise you to ask for more tasks, if you can handle them, and turn them around in the necessary time frame. You might not be given the most exciting work but you will still learn. A couple more tidbits…hmmm let's see. Be positive and always offer to help. Live off your parents for as long as you can. LOL. Show initiative, be proactive in trying to conjure up solutions that might further the cause of your internship. DRESS professionally. I like to dress up anyway... Communicate. Take your opportunity seriously because you never know the possibilities.

Holland: Take time to reflect on the many learning opportunities and to develop professional relationships with the helpful staff members and personal relationships with the other SURP students. Be sure to ask questions, the expert staff is an excellent resource as you grow as research student.

LOCAL

Supermarkets

Baker's Supermarkets
888 S Saddle Creek Rd
Omaha, NE 68106
(402) 551-0613

Wohlner's Grocery
3253 Dodge St
Omaha, NE 68131
(402) 551-6875

Pharmacies

Walgreens
225 N Saddle Creek Rd
Omaha, NE 68131
(402) 551-1797

CVS Pharmacy
4812 Dodge St
Omaha, NE 68132
(402) 558-2000

Transportation

Omaha Metro
http://www.metroareatransit.com/

Restaurants

Crave
200 S 31st Ave # 4103
Omaha, NE 68131
(402) 345-9999

Crescent Moon Ale House
3578 Farnam St
Omaha, NE 68131
(402) 345-1708

Smoke Pit Bbq & Lounge
230 S 25th St
Omaha, NE 68131
(402) 345-8681

Sam & Louie's New York Pizzeria
2416 Cuming St
Omaha, NE 68131
(402) 884-7773

California Tacos & More
3235 California St
Omaha, NE 68131
(402) 342-0212

Coffee Shop

Casa Blanca Cafe
3025 Farnam St
Omaha, NE 68131
(402) 884-3788

Sambuza Café
2425 Douglas St Ste 8
Omaha, NE 68131
(402) 346-6840

City Brew Coffee
540 N Saddle Creek Rd
Omaha, NE 68131
(402) 558-0028

Fast Food Restaurants

Wendy's
4308 Dodge St
Omaha, NE 68131
(402) 558-1111

McDonald's
122 S 40th St
Omaha, NE 68131
(402) 558-3300

Subway
4020 Dodge St
Omaha, NE 68131
(402) 551-1081
Arby's
4358 Dodge St
Omaha, NE 68131
(402) 556-9291

Burger King
4460 Dodge St
Omaha, NE 68131
(402) 554-0849

KFC
4358 Dodge St
Omaha, NE 68131
(402) 504-3474

Quiznos
417 N Saddle Creek Rd
Omaha, NE 68131
(402) 559-0000

Chick-Fil-A
3333 Farnam St
Omaha, NE 68131
(402) 351-4303

Runza
2952 Farnam St
Omaha, NE 68131
(402) 346-8551

Carryout/Delivery

Jimmy John's
107 N 40th St
Omaha, NE 68131
(402) 614-4545

Godfather's Pizza
2951 Farnam St
Omaha, NE 68131
(402) 341-5353

Pizza Hut
5024 Underwood Ave
Omaha, NE 68132
(402) 556-4000

China Taste
2420 Cuming St
Omaha, NE 68131
(402) 342-2288
Rice Bowl
505 N Saddle Creek Rd
Omaha, NE 68131
(402) 558-1222

Banks

Premier Bank
4440 Douglas St
Omaha, NE 68131
(402) 558-8000

Bank of the West
4444 Farnam St
Omaha, NE 68131
(402) 918-5690

U.S. Bank
520 N Saddle Creek Rd
Omaha, NE 68131
(800) 872-2657

Mutual Of Omaha Bank
3333 Farnam St # 10
Omaha, NE 68131

Movie Theaters

Marcus Midtown Theatre
3201 Farnam St
Omaha, NE 68131
(402) 346-6900

Dundee Theatre
4952 Dodge St
Omaha, NE 68132
(402) 551-3595

Aksarben Cinema
2110 S 67th St
Omaha, NE 68106
(402) 932-9858

Shopping

Crossroads Mall
7400 Dodge St
Omaha, NE 68114
(402) 397-0343

Westroads Mall
10000 California St
Omaha, NE 68114
(402) 397-2398

Campus Map


University of Nebraska Medical Center
Annex 14
Midwest Eye Care
BLUE PARKING
Clarkson Doctors Building North
Clarkson College
Clarkson Family Medicine
Clarkson College Apartments
Family Place
MMI Clinic
J.P. Lord School
Clarkson Doctors Building South
PURPLE PARKING
Durham Research Center
Munroe-Meyer Institute
Kiewit Tower
Durham Research Center 2
Swanson Hall
Storz Pavilion
Clarkson Tower
GREEN PARKING
Hixson-Lied Center
ORANGE PARKING
YELLOW PARKING
Durham Outpatient Center
Eppley Cancer Institute
Eppley Science Hall
University Tower
Poynter Hall
College of Nursing
Administration Center
Center for College of Nursing Sciences
Lot 50 Parking Structure
Specialty Services Pavilion
Lied Transplant Center
Witson Hall
Parking Lot 53 (ice rink parking)
College of Pharmacy
East Utility Plant
Shackleford Hall
Bennett Hall
University Eye Specialties
Center Utility Plant
Michael F. Sorrell Center for Health Science Education
Ice Rink
Potter House
Academic Research Services
Harold M. and Beverly Maurer Center for Public Health
Parking Lot 4
Parking Lot 15 (ice rink parking)
4230 Building
Alumni House
General Supply Warehouse
Parking Lot 39 (ice rink parking)
Student Life Center
Student Apartments
Ronald McDonald House
Weigel Williamson Center for Visual Rehabilitation
Home Instead Center for Successful Aging
Emergency
Dodge
Farnam
Leavenworth Street
Jones Street
Jackson Street
Harney Street
Center Avenue
40th Street
38th Avenue
39th Street
42nd
41st
Produced by MedMaps®
www.medmaps.com

Itinerary for Drs. Shafiq Khan & Valerie Odero-Marah
Clark Atlanta University

Monday, July 28, 2013

3:40 p.m. Arrive on American Airlines Flight 1286. Drs. Shafiq Khan & Valerie Odero-Marah will rent a car and drive themselves to the hotel. (Dr. Lin's Cell # 402-990-1662)

Hotel Reservations: Element Omaha Midtown Crossing
3253 Dodge Street
Omaha, NE 68131
402-614-8080
Dr. Shafiq Khan Reservation Number: 163144
Dr.Valerie Odero-Marah Reservation Number: 163145

Dinner with Drs. Chaney, Lin, Khan and Odero-Marah

Tuesday, July 29, 2013

8:30 a.m. Drs. Shafiq Khan & Valerie Odero-Marah will drive to UNMC

9:00 a.m. Dr. Batra, DRC1 7005
Drs. Shafiq Khan and Valerie Odero-Marah

9:45 a.m. Dr. Cheng, DRC1 7003 - **Dr. Shafiq Khan**
Dr. Melissa Teoh-Fitzgerald DRC1 5050 -**Dr. Valerie Odero-Marah**

10:30 a.m. Dr. Lin DRC1 7003-**Dr. Shafiq Khan**
Dr. Cheng, DRC1 7042 **- Dr. Valerie Odero-Marah**

11:15 a.m. Dr. Yaping Tu, Creighton University – DRCI 7003
Drs. Shafiq Khan and Valerie Odero-Marah

12:00 p.m. Lunch with CAU students and mentors-DRC1 6003

1:15 p.m. Dr. Odero-Marah - Prepare for seminar presentation

1:30 p.m. Seminar presentations– DRC1 1002
"Snail Regulation of Cathepsins can be Antagonized by Muscadine Grape Skin Extract in Prostate and Breast Cancer Cells"

3:00 p.m. Dr. Lin will drive Dr. Shafiq Khan to the airport
Valerie Odero-Marah depart for Cedar Rapids, IA

4:25 p.m. Dr. Shafiq Khan Depart on American Airlines 1286

Wednesday, July 30, 2013

9:45 a.m. Valerie Odero-Marah
Depart on American Airlines 3172
Cedar Rapids, IA

UNIVERSITY OF NEBRASKA MEDICAL CENTER unmc.edu

Seminar Series

DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

"Snail Regulation of Cathepsins can be Antagonized by Muscadine Grape Skin Extract in Prostate and Breast Cancer Cells"

"

TUESDAY, JULY 29, 2014

1:30 P.M.

DRCI, Amphitheater (1002)

Valerie Odero-Marah, Ph.D.
Assistant Professor
Department of Biological Sciences
Clark Atlanta University
Atlanta, Georgia

Special BMB Department Seminar

University of Nebraska Medical Center

Nebraska Prostate Cancer Research Program (NPCRP)

http://www.unmc.edu/biochemistry/index.cfm?L1_ID=48&CONREF=84

Funding:
Department of Defense
Prostate Cancer Research Program,
the Office of the Congressionally Directed Medical Research Programs (CDMRP)
PC094594, 2010/04/15 – 2014/04/14
PC121645, 2013/09/01 – 2015/08/31

Collaborations:
Dr. Ming-Fong Lin and Dr. William Chaney,
University of Nebraska Medical Center (UNMC),
and
Dr. Shafiq Khan and Dr. Valerie Odero-Marah,
Clark Atlanta University (CAU)

Supported by
Department of Biochemistry & Molecular Biology, UNMC
Nebraska Center for Functional Genomics,
NIH INBRE Program
Drs. Batra, Cowan, Turpen and Davies

Faculty members:
Creighton University (CU) at Omaha;
University of Nebraska – Lincoln (UNL) and
University of Nebraska Medical Center (UNMC)

Acknowledgements:

Research Mentors and Projects

Investigators	Institution	Projects
S. Batra	UNMC	Genetic Alterations in Prostate Cancer Progression
W. Chaney	UNMC	Glycobiology in Prostate Cancer
P. Cheng	UNMC	Glycomics in Prostate Cancer Metastasis and Gene Therapy
K. Datta	UNMC	Growth regulation and therapy of Prostate cancer
J. Davis	UNMC	Hormone Regulation of Tumor Cell Development
R. Lewis	UNMC	IGF Receptors in Prostate Cancer
M.-F. Lin	UNMC	Androgen Regulation of Prostate Cancer Growth and Development
P. Mehta	UNMC	Gap Junction Proteins in Prostate Cancer Metastasis
J. Mott	UNMC	MicroRNA in growth regulation and therapy
E. Rogan	UNMC	Metabolism of Dietary and Environmental Chemicals to Mutagenic and Genotoxic Species
M. Simpson	UNL	The Role of Hylauronate in Prostate Cancer Development
R. Singh	UNMC	Prostate Cancer Metastasis and Immunology
Y. Tu	CU	Regulation of G-Protein-Coupled Receptors in Prostate Cancer

Acknowledgements:

DOD CDMRP PCa Research Program
PC121645

Surinder **Batra**: Sierra Lawhorne

Pi-Wan **Cheng**: Brittney Carr

Ming-Fong **Lin**: Tashika Robinson

Parmender **Mehta**: Quentin Loyd

Supports:

BMB, UNMC

Ms. Amy Dodson, MBA
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Ms. Sue Klima

CCRTD/RCMI Program, CAU

Ms. Priscilla Bakari, MA

UNIVERSITY OF NEBRASKA MEDICAL CENTER unmc.edu

Seminar Series

DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

"Snail Regulation of Cathepsins can be Antagonized by Muscadine Grape Skin Extract in Prostate and Breast Cancer Cells"

"

TUESDAY, JULY 29, 2014

1:30 P.M.

DRCI, Amphitheater (1002)

Valerie Odero-Marah, Ph.D.
Associate Professor
Department of Biological Sciences
Clark Atlanta University
Atlanta, Georgia

Special BMB Department Seminar

University of Nebraska Medical Center

SURP Research Colloquium

Poster Session

Summer Undergraduate Research Program

August 7th , 2014

Truhlsen Campus Events Center

10:00 A.M. – 12:30 P.M.

Setup 9:00am – 9:45am

SURP Program Reception 1:00 – 2:00 p.m.

Storz Pavillion

All Participants and Mentors Are Invited

Sponsored by:

Biomedical Research Training Program, Eppley Institute, Office of Research and Development, and Recruitment and Student Engagement

Responses to Nebraska Prostate Cancer Research Scholars Program Evaluation Survey
- **A Summary**

1. How satisfied are you with the Nebraska Prostate Cancer Research Scholars Program (NPCRSP)?

Very Satisfied Satisfied Dissatisfied Very Dissatisfied

Very Satisfied
Very Satisfied
Very Satisfied
Satisfied

2. How did you originally learn about the NPCRSP?

I learned of NPCRSP through my Genetics professor at my school, Dr. Odero-Marah.

I learned about the NPCRSP through my advisor Dr. Valerie Odero-Marah of Clark Atlanta University as well as students who previously attended the program and spoke positively of the program.

I learned about the NPCRSP program through my Biology department at my institution.

I learned about the program from noticing the flyers that were up around the CAU research center. I also had a friend that did the internship as well, and she told me that it would be a great opportunity.

3. Did you have a clear set of expectations of the NPCRSP when you became a NPCR Scholar?

Yes. I expected to gain further competency in scientific research, specifically in prostate cancer.

My expectations upon being selected as a NPCR Scholar were to learn various new laboratory techniques, interact with other research scholars, and to take away as much knowledge as possible. Working in the biochemistry and molecular biology laboratory has given me a leg up in my cell and molecular biology course this semester.

Yes, I did.

Yes, I expected to gain a lot of research knowledge. I also expected to experience the feeling of how working in a real research lab would be, if I decided to enter graduate school. The program really helped me make up my mind about going to graduate school.

4. How would you rate your research experience as it relates to helping you make decisions related to your education and career plans?

My research experience has helped to become familiar with the realities/limitations of a scientific researcher. I am more confident in my decision to pursuit professional school upon completion of my undergraduate degree and to pursuit a career in oncology.

On a scale of 1-10, I would say the NPCRSP would be a firm 9 in regards to solidifying my decision to want to continue to do research as a part of my ultimate career goals simply for the challenge it constantly posed to allow me to always think critically.

On a scale of 1-10 with 10 being the greatest experience and 1 being the worst experience, I would rate my overall research experience at a 9 as it relates to my education and my career plans.

On a scale of 1- 10, I rate the experience a 9. This experience really helped me decide that I would like to go to graduate school after I graduate next year.

Alcohol Induction of Golgi Fragmentation and Prevention of Golgi Targeting of Selective Glycosyltransferases in Androgen Sensitive Prostate Cancer Cells

Britney Carr[1], Armen Petrosyan[2,3] , Ganapati Bhat [2,3] and Pi-Wan Cheng[2,3,4]

[1]Clark Atlanta University, Atlanta, GA; [2]VA Nebraska Western Iowa Health Care System, Omaha, NE; [3]Department of Biochemistry and Molecular Biology, College of Medicine; and [4]Pamela & Fred Buffett Cancer Center, Omaha, NE

Background

• Prostate cancer is the second leading cause of cancer death among men older than 55 in the United States (1). During the early stages of prostate cancer development, androgen promotes tumor growth. Therefore, androgen-ablation therapy has been developed for treating these patients. However, androgen-refractory will develop several years later. Once this occurs, the cancer is incurable. Therefore, understanding how androgen-sensitive prostate cancer is developed into androgen-refractory could aid the development of effective therapy.

• It was reported (2) that androgen-sensitive LNCaP prostate cancer cells are susceptible while the androgen-refractory PC3 and DU145 cells are resistant to galectin 1-induced apoptosis. We found that the Golgi in PC3 and DU145 cells was fragmented but the Golgi in LNCaP cells was not. Also, loss of giantin function in advanced prostate cancer cells resulted in failure of core 2 N-acetylglucosaminyltransferase L (C2GnT-L) to target the Golgi and the inability of these cells to synthesize core 2-associated polylactosamine, the target of galectin 1 (**Fig. 1**). Inhibition or knockdown of non-muscle myosin IIA renders these cells susceptible to galectin 1-induced apoptosis by restoring giantin function, compact Golgi morphology, Golgi targeting of C2GnT-L, and formation of core 2-associated polylactosamine (3).

• Excessive alcohol consumption can increase the risk of developing cancer, including head and neck, esophagus, liver, breast and colorectal (4). However, there is no consensus about the effect of alcohol consumption on prostate cancer (5-7). Because alcohol treatment causes Golgi fragmentation in neuronal cells (8), which we also confirmed in hepatocyes and airway epithelial cells, it is of interest to see if alcohol treatment of LNCaP cells can cause Golgi fragmentation and prevent Golgi targeting of C2GnT-L. If it does, this may be used as a model for studying the progression of prostate cancer from androgen-sensitive to androgen-refractory.

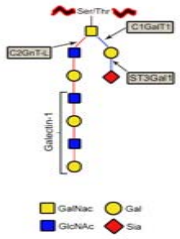

Figure 1. Schema of selective mucin core 1 and core 2-associated O-glycans in mammalian cells. The core 1 O-glycan is generated by C1GalT1. ST3Gal1 and C2GnT-L compete for the core 1 substrate to generate Siaα2-3Galβ1-3GalNAc and Galβ1-3(GlcNAcβ1-6)GalNAc, respectively. GlcNAc can be extended by a polylactosamine (Gal-GlcNAc) sequence to render these cells susceptible to galectin 1-induced apoptosis (4).

Objective

The goal of this study was to determine if alcohol treatment of androgen -sensitive prostate cancer cells can result in Golgi fragmentation and prevent Golgi targeting of C2GnT-L

Experimental Methods

• Cell culture and ethanol treatment: Androgen-sensitive prostatic normal (RWPE-1) and cancer (LNCaP) cells were cultured in RPMI medium containing 10% FBS and with or without 25 mM ethanol (**Fig. 2**) for 5 days. The medium was replaced every 12 h.

• Confocal immunofluorescence microscopic analysis of Golgi morphology and localization of glycosyltransferases: The cells were cultured in cover slips overnight and then treated with Ab against giantin and then red-fluorescence-tagged secondary Ab and Ab against either ST3Gal1 or C2GnT-L followed by green-fluorescence-tagged secondary Ab. Fragmented Golgi (< 2 μ) was measured (**Fig. 3**).

• Western blot analysis of giantin: Giantin in the homogenates of RWPE-1 and LNCaP cells with and without ethanol treatment was separated by SDS-PAGE and probed with giantin Ab and then HRP-secondary Ab following transfer to nitrocellulose membrane. *ß*-actin is used for normalization of samples

• Quantitative real-time PCR analysis of alcohol metabolizing enzymes: mRNAs of RWPE-1 and LNCaP cells were converted to cDNAs, which were used for measuring the gene expression profiles of alcohol dehydrogenase enzymes (ADH1A-C and CYP2E1) and acetaldehyde dehydrogenases (ALDH1A & 2) by quantitative PCR. The results are expressed as relative amounts of each of these genes relative to that of GAPDH.

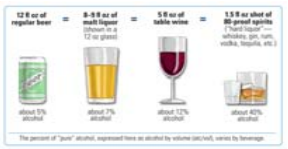

Figure 2. Ethanol concentration (25 mM) used in this study represents the legal blood alcohol limit (0.08%), which is equivalent to approximately 2 servings of alcohol beverages for females and 3-4 servings for males.

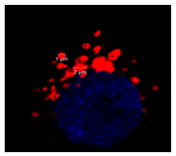

Figure 3. Determination of the Golgi fragments based on the sizes (<2μm)

Results

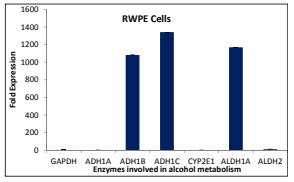

Figure 4. Gene Expression levels of Alcohol Dehydrogenases (1A-C), CYP2E1, and acetaldehyde dehydrogenases (1A&2) relative to that of GAPDH (=1.0) in RWPE-1 cells: ADH>ALDH, suggesting accumulation of acetaldehyde, a reactive toxic metabolite.

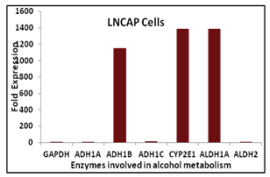

Figure 5. Gene expression levels of alcohol dehydrogenases (1A-C), CYP2E1, and acetaldehyde dehydrogenases (1A & 2) relative to that of GAPDH in LNCaP cells: ADH+CYP2E1 > ALDH suggesting accumulation of acetaldehyde.

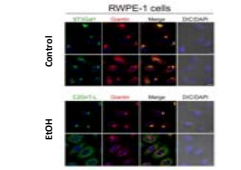

Figure 6. Ethanol treatment of RWPE-1 cells induces Golgi fragmentation and prevents Golgi targeting of C2GnT-L but not ST3Gal1.

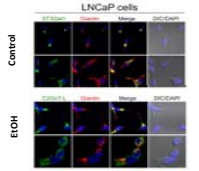

Figure 7. Ethanol treatment of RWPE-1 cells induces Golgi fragmentation and prevents Golgi targeting of C2GnT-L but not ST3Gal1.

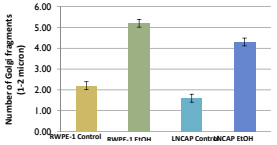

Figure 8. Quantification of Golgi fragments in RWPI and LNCaP cells treated with ethanol: Alcohol treatment induces Golgi fragmentation.

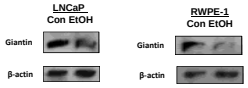

Figure 9. Western blots of giantin in RWPE-1 and LNCaP cells without and with ethanol treatment: Alcohol treatment reduces giantin.

Conclusions

1) **Alcohol abuse may confer a survival advantage to androgen -sensitive prostate cancer to promote progression .**

2) **Alcohol treatment of androgen –sensitive prostate cancer cells may be used as a model for studying the progression of prostate cancer from androgen -sensitive to androgen -refractory .**

Acknowledgements

This work is supported by PC121645 from PCRP of DoD CDMRP " The Nebraska Prostate Cancer Research Program " and a State of Nebraska LB506 grant. LNCaP cells were provided by Dr. Ming-Fong Lin at the University of Nebraska Medical Center, Omaha, NE.

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Appendix #24:

Quentin Loyd, Ray, A., Katoch, P., Kelysey, L., and **Parmender Mehta**. Regulation of gap junction protein in prostate cancer cells. UNMC Summer Undergraduate Research Program Poster. August 7, 2014.

- The poster is very big with size over 35Mb in pdf file. It is too big to be included in the file.

Inhibition of SHH signaling enhances Docetaxel efficacy in castration-resistant prostate cancer cells

Sierra L. Lawhorne[1,2], Sakthivel Muniyan[1], Parthasarathy Seshacharyulu[1], Ming-Fong Lin[1,3], and Surinder K. Batra[1,3]

[1]*Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, Nebraska*

[2]*Department of Biology, Clark Atlanta University, Atlanta, Georgia*

[3]*Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, Nebraska*

Abstract

Docetaxel, the first line chemotherapeutic agent, has improved the survival in men with metastatic castration-resistant prostate cancer (CR PCa). However, most patients often develop resistance and limited options are available. Therefore, more effective treatment strategies are needed to improve the overall survival. Emerging studies have shown the involvement of self renewal molecules and signaling pathways in the development, progression and therapeutic resistance of PCa. We and others previously demonstrated that sonic hedgehog (Shh) ligand is significantly upregulated in CR PCa cells when compared to normal epithelial cells. In the present study, we have shown that both GDC-0449 and Docetaxel dose dependently inhibited the LNCaP C-81 cell proliferation. The results have indicated that the combination treatment of GDC-0449 and Docetaxel induced higher anti-proliferative effects on human CR PCa cells than the drugs alone. Indeed, exposure of LNCaP C-81 cells to the IC_{50} concentration of GDC-0449 enhanced sensitivity to conventional chemotherapeutic agent Docetaxel. Mechanistically, treatment of LNCaP C-81 cells with GDC-0449 and Docetaxel significantly inhibited ABCG2 protein expression level. Our findings suggest that targeting Shh-signaling could enhance the docetaxel activity and may represent a novel approach for treatment of CR PCa.

Background

Prostate cancer (PCa) is the second leading cause of cancer-related deaths among men in the U.S. in 2014, it is projected to be approximately 233,000 new cases and 29,480 deaths (1). Although androgen deprivation therapy and Docetexel are considered as effective treatment options for metastatic and castration-resistant (CR) PCa, respectively, most PCa cases often recur and/or develop resistance. Patients acquired resistance to Docetaxel is commonly fatal and treatment of Docetaxel-resistant patients remains a critical clinical challenge. *In vitro* studies revealed that the Docetaxel-resistance phenotype corresponds to a small, intrinsically multidrug resistant subpopulation present in unselected CR PCa cells. Emerging studies have also shown that tumor-initiating cells may preferentially survive exposure to chemotherapy; and hypothesized that the pathways involved in the regulation of stem cell renewal may play an vital role in the maintenance of tumor initiating cells (2,3).

The Hedgehog (Hh) signaling pathway is playing a vital role during embryonic development including cell growth, cell differentiation, patterning, organogenesis and is involved in stem cell population maintenance, tissue repair and regeneration (4). In PCa, uncontrolled activation of the Hh signaling pathway has been observed and emerging evidence shows that Hh signaling plays a crucial role in the development as well as in the progression of this disease to more aggressive and even therapy-resistant disease states (5). These studies strongly implicate aberrant Hh signaling as a potentially viable therapeutic target for management of advanced PCa. In this study, we have shown benefit of using GDC-0449 to enhance the apoptotic effects of first line chemotherapeutic drug, Docetaxel on CR PCa cells.

Hypothesis

Inhibition of sonic hedgehog signaling by GDC-0449 would enhance the efficacy of Docetaxel in castration-resistant prostate cancer cells.

Methods

MTT assay was performed to determine the IC50 value of Docetaxel and GDC-0449 and cell proliferation.

Western Blot analysis was performed to evaluate the Shh expression level, pro- and anti-apoptotic protein and drug resistant protein ABCG2 expression levels on LNCaP C-81 cells.

Results

Effect of Docetaxel and GDC-0449 on LNCaP C-81 cell viability

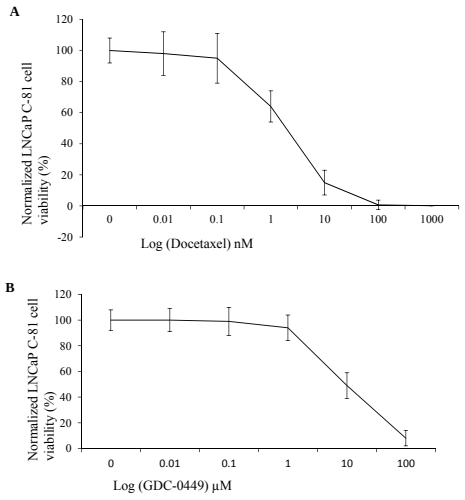

Figure 1: Dose-response curves of LNCaP C-81 cells for log concentrations of Docetaxel (A) and GDC-0449 (B). Cells were exposed to drugs for 72 hours and IC50 values were determined. Observations were normalized to a DMSO control, and represented here as 100% viability. Data represent (mean ± SEM) values of three independent experiments.

Results

SHH inhibitor GDC-0449 increases the growth suppressive effect of Docetaxel in the LNCaP C-81 PCa cells

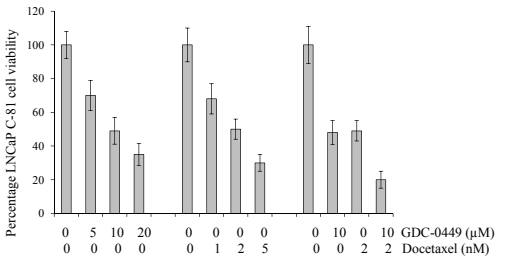

Figure 2: LNCaP C-81 cells were treated for 72 hours with different concentrations of GDC-0449 and Docetaxel alone or in combination, for three days and the cell proliferation was evaluated by MTT assay.

Combined activity of GDC-0449 and Docetaxel decrease Shh and cyclin D1 level in the LNCaP C-81 PCa cells

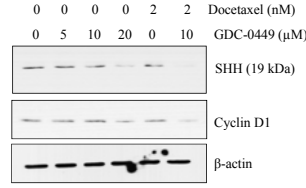

Figure 3: Western blot analyses showed decreased Shh and cyclin D1protein expression level in GDC-0449 and Docetaxel treated cells compared to untreated cells.

SHH inhibitor GDC-0449 improve the apoptotic effect of Docetaxel in the LNCaP C-81 PCa cells

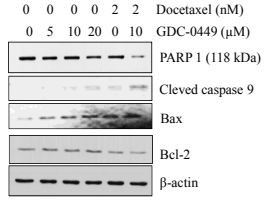

Figure 4: Effect of GDC-0449 and Docetaxel on PARP-1, cleaved caspase-9, Bax, Bcl2 protein expression level. B-actin were detected as loading control.

Results

SHH inhibitor GDC 0449 combined with Docetaxel suppresses multi-drug resistant protein ABCG2 on LNCaP C-81 Pca cells

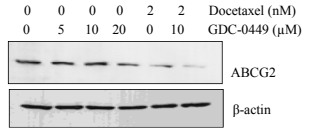

Figure 5: Western blot analysis demonstrating decreased ABCG2 protein expression level on GDC-0449 and Docetaxel treated LNCaP C-81 cells.

Summary

This study presents indicate that inhibition of Shh-signaling pathway by GDC-0449 improves the activity of Docetaxel in CR PCa cells by

- Inhibiting the Shh signaling
- Inducing apoptosis
- Inhibiting the multi-drug resistant protein ABCG2.

Hence, these data suggest that GDC-0449 can be used as a single or in combination with chemotherapeutic agent Docetaxel for improving the survival of metastatic CR PCa patients.

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Acknowledgements

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Inhibition of Tumorigenicity of Castration-Resistant Human Prostate Cancer Cells by Imidazopyridine Derivatives

Tashika Robinson[1], Matthew Ingresoll[2], Sakthivel Muniyan[2], Marisha Morris[1], Kyle Hoelting[2], Xiu R. Bu[1], Surinder K. Bartra[2], Ming-Fong Lin[2],
[1] Clark Atlanta University, Atlanta, GA; [2] University of Nebraska Medical Center, Omaha, NE

ABSTRACT

Background: Prostate adenocarcinoma is the most common cancer affecting men in the United States. Androgen deprivation therapy (ADT) such as castration or casodex androgen receptor inhibitor is the current standard treatment for metastatic prostate cancer (PCa). However, most prostate cancers relapse and develop the castration-resistant (CR) phenotype. These tumors tend to be very aggressive and metastasize to different loci of the body. There is no effective treatment for CR PCa. Imidazopyridine derivatives are a new class of compounds that have been shown to be effective against CR PCa. In this study we investigated the anti-tumorgenic effect of four novel imidazopyridine derivatives against CR PCa. Our results show these compounds effectively inhibit CR PCa cell growth and warrant further investigation as promising therapeutic agents.
Materials & Methods: In this study four imidazopyridines were synthesized and investigated to determine their ability to inhibit prostate cancer cell proliferation and tumorigenicity. The compounds: HIMP, M-MeI, OMP, and EtOP were synthesized by Dr. Bu's laboratory of Clark Atlanta University. Androgen independent (AI) human adenocarcinoma cell lines LNCaP C-81, Neuroendocrine NE-1.3, MDA PCa2b AI, and normal prostate epithelium RWPE1 cells were used to study the compounds. DMSO was used as a negative control while previously classified DME was used as a positive control. Proliferation assays were conducted on C-81, NE-1.3, MDA PCa2b AI, and RWPE1 cells in the presence of 10-µM imidazopyridines inhibitors. Dosage assays were used to determine the IC_{50} of each compound using C-81 cells. To observe the effect of the inhibitors over time, a kinetic proliferation study was also conducted. Additionally, we used the clonogenic and soft agar assays to determine the compounds' ability to inhibit tumorigenicity in a 2-dimensional and 3-dimensional environment respectively. Immunoblot analysis was carried out to establish which biochemical pathways were being affected by the imidazopyridine derivatives.
Results: Of the four compounds investigated, M-MeI was found to be the most potent inhibitor of tumorigenicity, followed by EtOP and OMP with HIMP having the least effect. M-MeI was more effective than positive control DME at inhibiting cell proliferation in regular conditions, cell adherence, and tumorigenicity. We observed down-regulation of PIK3 by HIMP and M-MeI. Apoptosis inhibitor survivn and AR protein level were decreased in the presence of all four compounds as were proliferation markers Cyclin B1 and Cyclin D1. Correspondingly, protein levels of apoptosis inducer p53 were increased. Our results demonstrate the need for further exploration of the imidazopyridine derivatives as potential therapeutic agents for CR PCa treatment.

INTRODUCTION

Prostate cancer is the most common adenocarcinoma in men and is the second leading cause of cancer-related death in males in the United States. It is predicted 1 in 7 men will develop prostate cancer. Prostate cancer deaths are usually the result of metastatic castration-resistant prostate cancer (CR PCa), and historically the average survival for men with CR PCa has been less than two years. Classical treatments such as ADT are ineffective at treating CR PCa, and at this time CR PCa remains an incurable disease (Kirby et al., 2011).

Imidazopyridine derivatives are an aromatic aldehyde and a pyridine group that have been demonstrated to possess anti-tumor activity. Previously studied DME is an imidazopyridine derivative, which has been shown to exhibit anti-proliferative activity, inhibit cell migration, and induce apoptosis in CR PCa. This is achieved in part by inhibition of phosphoinositide 3-kinase (PI3K) signaling, which plays a critical role in regulating cell growth, differentiation, drug resistance, and survival. (Muniyan et al., 2014). DME was used as a positive control in this study and while we investigated four novel imidazopyridine derivatives: HIMP, M-MeI, OMP, and EtOP to see how they influence PCa cell proliferation and tumorigenicity and also better efficacy than DME.

LNCaP C-81 cells exhibit CR phenotype: functional androgen receptor expression, prostate –specific antigen (PSA) secretion with rapid cell growth in steroid reduced condition. MDA PCa2b-AI cells also exhibit CR PCa phenotype and were used to confirm results demonstrated by LNCaP C-81 cells. NE cells are a group of cells positioned along luminal epithelial cells and basal cells of the prostate gland. NE cells are also present in PCa. Many studies have shown neuroendocrine (NE) cells promote tumorigenicity of PCa cells under androgen deprivation conditions (Yuan et al., 2006). Therefore, NE 1-3 cells were used to investigate the efficacy of the imidazopyridine derivatives. We also investigated RWPE1 cells, which are normal epithelial prostate cells to demonstrate the toxicity on non-tumorigenic cell lines. Imminent mechanistic research on these compounds will provide a new therapeutic approach to treat relapsed patients.

RESULTS

Imidazopyridine Derivatives Structures

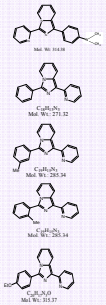

Fig 1. Molecular structure of Imidazopyridine Derivatives investigated.

Dosage Effect of Imidazopyridine Derivatives on C-81 Cells under Regular Conditions

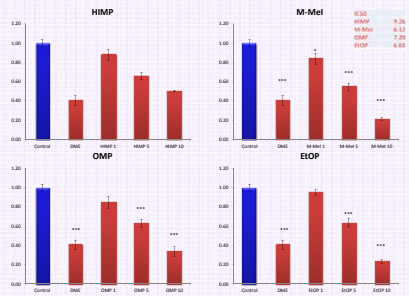

Fig 2. Dosage assays were used to determine the IC_{50} of each compound on C-81 cells. M-MeI was observed to have the best efficacy on growth followed by EtOP, OMP, and HIMP. *P < 0.05, **P < 0.005, ***P < 0.0005.

Dosage Effect of Imidazopyridine Derivatives on C-81 Cells under Steroid-Reduced Conditions

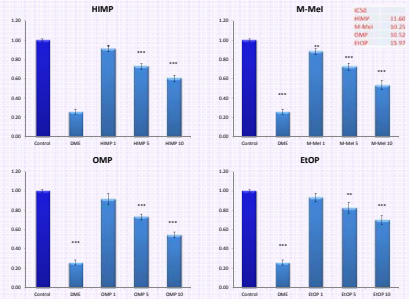

Fig 3. Dosage assays were used to determine the IC_{50} of each compound on C-81 cells in steroid-reduced conditions. M-MeI was observed to have the best efficacy on growth suppression in steroid-reduced conditions followed by OMP, HIMP, and EtOP. *P < 0.05, **P < 0.005, ***P < 0.0005.

RESULTS

The Effect of Imidazopyridine Derivatives on Various Benign and PCa Cells

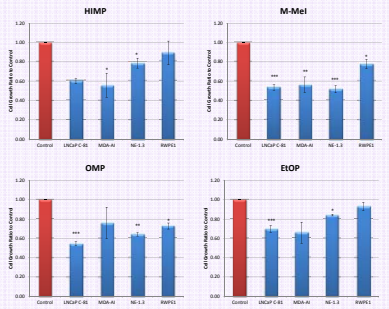

Fig 4. Proliferation assays were used to determine proliferation inhibition efficacy of investigated compounds on LNCaP C-81, NE-1.3, MDA PCa2b, and RWPE1 cells . M-MeI showed the most inhibition on PCa cells. *P < 0.05, **P < 0.005, ***P < 0.0005.

Kinetic Effect of Imidazopyridine Derivatives on C-81 Cells

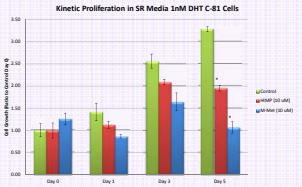

Fig 5. Kinetic proliferation assays were used to investigate the cell growth inhibition by HIMP and M-MeI in steroid-reduced conditions. Statistical analysis shows that in SR conditions, control cell continuously grew on day 3 and 5, while M-MeI exhibited better efficacy than HIMP. *P < 0.05, **P < 0.005, ***P < 0.0005.

The Effect of Imidazopyridine Derivatives on Tumorigenicity on C-81 Cells in a 3-Dimensional Environment

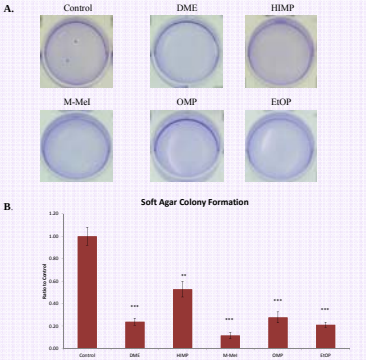

Fig 6. Soft agar assay was used to determine the effect of imidazopyridine derivatives on tumorigenicity of C-81 cells. **A.** Inhibition of tumorigenicity in a 3-dimensional environment. **B.** Statistical analysis on the ratio of the investigated compounds to the control. *P < 0.05, **P < 0.005, ***P < 0.0005.

RESULTS

The Effect of Imidazopyridines on Tumorigenicity of C-81 Cells in a 2-Dimensional Environment

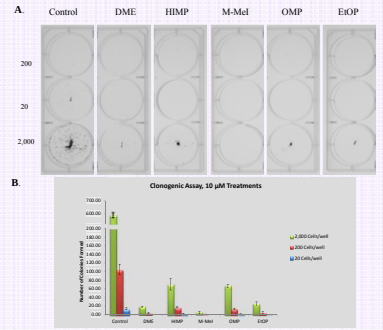

Fig 7. Clonogenic assay was used to determine the effect of imidazopyridine derivatives on colony formation of C-81 cells. **A.** Inhibition of tumorigenicity in a 2-dimensional environment. **B.** Quantitative analysis showed M-MeI has the best efficacy on tumorigenicity in a 2-dimensional environment. All values are significant.

Molecular Profiling on Imidazopyridine- treated CR PCa Cells

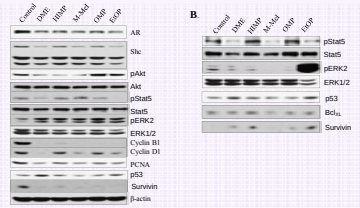

Fig 8. Immunoblot analyses were used to investigate pathways affected by imidazopyridines HIMP, M-MeI, OMP, and EtOP. **A**. LNCaP C-81 cells – SR medium. **B**. MDA PCa2b AI cells – SR medium.

DISCUSSION

The imidozopyrydine derivatives HIMP, M-MeI, OMP, and EtOP were investigated in order to determine their efficacy on various benign and PCa cells. Experimental assays determined that M-MeI exhibited the highest inhibition efficacy on both tumorigenicity and cell proliferation. PIK3 signaling which plays a critical role in regulating cell growth, differentiation, drug resistance, and survival was down regulated by HIMP and M-MeI. M-MeI showed greater differential effect between benign and PCa cells as determined by proliferation assays. Further investigation will analyze the efficacy of imidozopyrydine derivatives on various benign and PCa cells on cell migration, adhesion, and their potential for treating CR PCa.

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PLOS | ONE

RESEARCH ARTICLE

Novel Imidazopyridine Derivatives Possess Anti-Tumor Effect on Human Castration-Resistant Prostate Cancer Cells

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Abstract

Prostate cancer (PCa) is the second leading cause of cancer-related death afflicting United States males. Most treatments to-date for metastatic PCa include androgen-deprivation therapy and second-generation anti-androgens such as abiraterone acetate and enzalutamide. However, a majority of patients eventually develop resistance to these therapies and relapse into the lethal, castration-resistant form of PCa to which no adequate treatment option remains. Hence, there is an immediate need to develop effective therapeutic agents toward this patient population. Imidazopyridines have recently been shown to possess Akt kinase inhibitory activity; thus in this study, we investigated the inhibitory effect of novel imidazopyridine derivatives HIMP, M-MeI, OMP, and EtOP on different human castration-resistant PCa cells. Among these compounds, HIMP and M-MeI were found to possess selective dose- and time-dependent growth inhibition: they reduced castration-resistant PCa cell proliferation and spared benign prostate epithelial cells. Using LNCaP C-81 cells as the model system, these compounds also reduced colony formation as well as cell adhesion and migration, and M-MeI was the most potent in all studies. Further investigation revealed that while HIMP primarily inhibits PCa cell growth via suppression of PI3K/Akt signaling pathway, M-MeI can inhibit both PI3K/Akt and androgen receptor pathways and arrest cell growth in the G2 phase. Thus, our results indicate the novel compound M-MeI to be a promising candidate for castration-resistant PCa therapy, and future studies investigating the mechanism of imidazopyridine inhibition may aid to the development of effective anti-PCa agents.

data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Introduction

Prostate cancer (PCa) remains the most commonly diagnosed solid tumor and the second leading cause of cancer-related death in United States men, maintaining a need for new effective treatment options [1]. Currently, androgen-deprivation therapy (ADT) is the standard course of treatment for metastatic PCa, however, most PCa patients relapse within 1–3 years and develop castration-resistant (CR) PCa which is unresponsive to ADT [2,3,4]. In 2004, a combination of docetaxel and prednisone was shown to increase patient median survival by 2–3 months, making it the standard-of-care treatment for CR PCa [5]. Recently, the FDA has approved additional compounds such as novel taxane chemotherapeutic cabazitaxel [6], androgen synthesis inhibitor abiraterone acetate [7], AR signaling inhibitor enzalutamide [8], immunotherapeutic sipuleucel-T [9], and bone micro-environment-targeted radiopharmaceutical alpharadin (Radium-223) for treating CR PCa [10]. However, these treatment options are only able to prolong survival by a few months and the average period of CR PCa patient survival remains less than two years [11]. Despite advancements in post-ADT treatment strategies, CR PCa remains an incurable disease; thus there is a great need for alternative therapeutic options.

While androgen insensitivity can be manifested in multiple ways; one proposed alternative mechanism is the increased activation of Akt signaling under androgen deprived conditions. Akt is known to regulate cell cycle, metabolism, angiogenesis, and cell survival in PCa and its activation may contribute to tumor resistance to ADT and anti-androgens [12,13]. One mechanism through which Akt may contribute to PCa survivability is via modulation of androgen receptor (AR) signaling. In addition to inducing cell growth, AR also has a role in regulating apoptosis. Upon phosphorylation of AR at Ser-210 and Ser-790 by Akt, AR-mediated apoptosis is suppressed. Through this mechanism, enhanced Akt activity in PCa may contribute to PCa survivability upon ADT [13]. Indeed, genetic loss and/or mutations in the phosphatidylinositol-3 kinase (PI3K)/Akt pathway that lead to signal deregulation may present in up-to 42% of primary prostate tumors and over 90% of metastatic tumors, making it a priority next-in-line therapeutic target [14]. Recently, investigations into imidazopyridines, a novel class of compounds containing aromatic aldehydes and a pyridine group, have demonstrated these compounds possess potent Akt kinase inhibitory activity [15–17]. Data shows these compounds have an anti-proliferative effect against CR PCa cells with the ability to simultaneously inhibit AR and PI3K/Akt/mTOR signaling pathways, making them promising therapeutic agents [18].

To investigate imidazopyridines' efficacy for PCa therapy, the LNCaP progressive cell model, originally characterized in Lin et. al. *JBC* 1998, was used as the primary cell model in this study. LNCaP C-81 cells are androgen-independent (AI), express prostate-specific antigen (PSA) in the absence of androgens, and gain the ability to synthesize testosterone from cholesterol under steroid-reduced (SR) conditions [19–22]. C-81 cells also possess enhanced proliferation, ability to form colonies, and migratory potential [21,23]. Most Importantly, LNCaP C-81 cells retain AR expression and correspond to the expression of AR in the majority of PCa as well as advanced CR PCa [19]. This makes them a superior cell model for therapeutic studies when compared to many other PCa cell lines. Other cell lines selected for this study include MDA PCa2b-AI, PC-3, and RWPE1. Upon passage, MDA PCa2b cells behave similarly to LNCaP cells and shift from androgen-sensitive (AS) at low passage to AI at high passage. MDA PCa2b-AI (MDA-AI) cells also retain AR expression and possess enhanced tumorgenicity; this makes MDA-AI and LNCaP C-81 preferable cell models for studying prostate adenocarcinoma. Further, due to the ability of imidazopyridine derivatives to target both Akt and AR pathways, it is prudent to investigate the compounds' effects on AR-negative PC-3 cells to

determine their efficacy in cells which lack classic androgen signaling mechanisms. In addition, PC-3 cell lines are more representative of small-cell neuroendocrine carcinoma than more clinically predominant adenocarcinoma [24]; therefore this cell line should be used in conjunction with models such as LNCaP and MDA PCa2b cell lines to expand clinical utility. Finally, immortalized benign prostate epithelium RWPE1 cells act as a control to gauge the selectivity of the imidazopyridine derivative compounds. Thus our cell models clearly represent the majority of molecular events observed in clinical implementations of modern PCa therapies.

Our results demonstrate these imidazopyridine derivatives are able to suppress human PCa cell proliferation in a dose- and time-dependent manner. Importantly, compound M-MeI exhibited selective potency against CR PCa cell proliferation in comparison to benign prostate epithelial cells. Furthermore, this compound was also found to inhibit cell migration, adhesion, and *in vitro* tumorigenicity. Our data is the first to demonstrate the anti-tumor effect of novel imidazopyridine derivatives HIMP, M-MeI, OMP, and EtOP on CR PCa cells and indicates M-MeI to be a promising lead therapeutic agent for future studies.

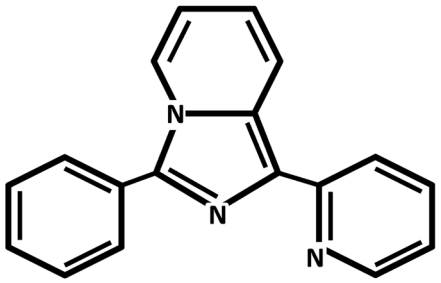
Materials and Methods

Materials

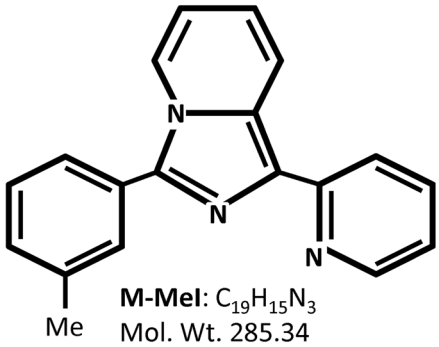
RPMI 1640 medium, Keratinocyte SFM medium, gentamicin, and L-glutamine were purchased from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) and charcoal-treated FBS were obtained from Atlanta Biologicals (Lawrenceville, GA). Molecular biology-grade agarose was procured from Fisher Biotech (Fair Lawn, NJ). Protein molecular weight standard markers, acrylamide, and Bradford protein assay kit were purchased from Bio-Rad (Hercules, CA). Polyclonal antibodies (Abs) recognizing all three isoforms of Shc protein (#29807, 1:4000) were purchased from Upstate (Lake Placid, NY). Anti-AR (#C1411, 1:400), anti-cyclin B_1 (#K1907, 1:1000), anti-cyclin D_1 (#A2712, 1:1000), anti-Bcl_{XL} (#F111, 1:1000), anti-Bax (#G241, 1:1000), anti-PCNA (#G261, 1:3000), anti-p53 (#K2607, 1:1000), anti-PSA (#E1812, 1:2000), anti-Survivin (#C271, 1:2000), and horseradish peroxidase-conjugated anti-mouse (#C2011, 1:5000), anti-rabbit (#D2910, 1:5000), anti-goat (#J0608, 1:5000) IgG Abs were all obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-Akt (Ser473) (#GA160, 1:1000), anti-Akt (#C1411, 1:2000), Anti-phospho-Stat5 (Y694) (#9351S, 1:4000), and anti-Stat5 (#9363, 1:2000) Abs were from Cell Signaling Technology (Beverly, MA). Anti-β-actin (#99H4842, 1:10000) Abs and 5α-dihydrotestosterone (DHT) were procured from Sigma (St. Louis, MO). Imidazopyridine derivatives HIMP (3-phenyl-1-(pyridine-2-yl)imidazo[1,5-*a*]pyridine), M-MeI (1-(pyridine-2-yl)-3-(*m*-tolyl)imidazo[1,5-*a*]pyridine), OMP (1-(pyridine-2-yl)-3-(*o*-tolyl)imidazo[1,5-*a*]pyridine), and EtOP (3-(4-ethoxyphenyl)-1-(pyridine-2-yl)imidazo[1,5-*a*]pyridine) were synthesized and provided by Dr. Xiu Bu as previously described [18,25] and their structures are shown in Fig 1. For ease of reading, chemical abbreviations are used throughout the text.

Cell Culture

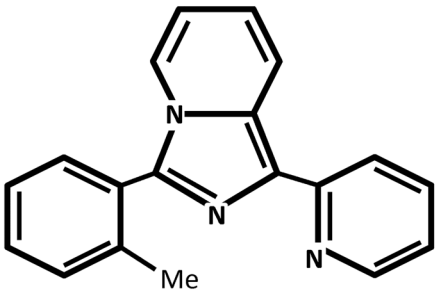
Human prostate carcinoma cell lines LNCaP, MDA PCa2b, PC-3, and immortalized benign prostate epithelial RWPE1 cells were originally obtained from the American Type Culture Collection (Rockville, MD, USA). LNCaP and PC-3 cells were routinely maintained in RPMI 1640 medium containing 5% FBS, 2 mM glutamine, and 50 μg/ml gentamicin [19,21]. MDA PCa2b cells were maintained in BRFF-HPC1 medium containing 20% FBS, 2 mM glutamine and 50 μg/ml gentamicin [26,27]. As reported previously, the LNCaP progressive cell model was established in which LNCaP cells at or below passage 33 are designated as C-33 and those at or greater than passage 81 as C-81. While C-33 cells are sensitive to androgen-induced growth, C-



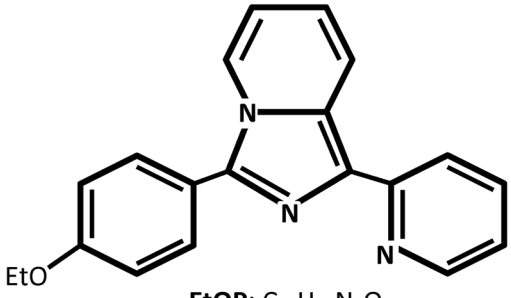
HIMP: $C_{18}H_{13}N_3$
Mol. Wt. 271.32



M-MeI: $C_{19}H_{15}N_3$
Mol. Wt. 285.34



OMP: $C_{19}H_{15}N_3$
Mol. Wt. 285.34



EtOP: $C_{20}H_{17}N_3O$
Mol. Wt. 315.37

Fig 1. Structures of imidazopyridine derivatives. HIMP, 3-phenyl-1-(pyridine-2-yl)imidazo[1,5-*a*]pyridine; M-MeI, 1-(pyridine-2-yl)-3-(*m*-tolyl)imidazo[1,5-*a*]pyridine; OMP,1-(pyridine-2-yl)-3-(*o*-tolyl)imidazo[1,5-*a*]pyridine; EtOP, 3-(4-ethoxyphenyl)-1-(pyridine-2-yl)imidazo[1,5-*a*]pyridine.

doi:10.1371/journal.pone.0131811.g001

81 cells are AI, express higher basal levels of PSA, and gain the ability to synthesize testosterone from cholesterol [19–22]. Similar to the LNCaP model, upon passage, MDAPCa2b cells become AI and possess many biochemical properties of clinical CR PCa, including the expression of functional AR, PSA secretion, and rapid cell proliferation in androgen-deprived conditions [19,21,22,27,28]. RWPE1 cells were cultured in Keratinocyte-SFM supplemented with bovine pituitary extract (25 μg/ml) and recombinant epidermal growth factor (0.15 ng/ml) along with 50 μg/ml gentamicin.

To mimic conditions of clinical ADT, cells were maintained in SR conditions, i.e., phenol red-free RPMI 1640 medium containing 5% charcoal/dextran-treated FBS, 2 mM glutamine, and 50 μg/ml gentamicin plus 1 nM DHT. Imidazopyridine derivatives HIMP, M-MeI, OMP, and EtOP were dissolved in dimethyl sulfoxide (DMSO) at 20 mM stock concentrations, stored at -20°C and diluted as needed for experimental conditions in the respective medium.

Cell Proliferation Assays

For cell proliferation experiments under regular conditions, LNCaP C-81 and MDA PCa2b-AI cells were seeded in regular culture medium and allowed to grow for 3 days, then changed to medium containing the respective compound and cultured for an additional 3 days. To determine cell proliferation under SR conditions, LNCaP C-81, MDA PCa2b-AI, PC-3, and RWPE1 cells were seeded in regular conditions and allowed to grow for 3 days. Cells were then steroid starved for 48 hours in SR medium and changed to fresh SR medium containing the respective compound, then cultured for an additional 3 days. Control groups received solvent DMSO alone. At the specified time point, cells were trypsinized and live cell numbers were counted via Trypan Blue Exclusion assay using a Cellometer Auto T4 Image-based cell counter (Nexcelom, MA, USA).

Cell Growth Kinetic and Dosage Determinations

Dose-dependent assays were conducted on LNCaP C-81 cells in the same manner as cell proliferation assays and used medium containing 0, 1, 5, or 10 μM of specified compound. To determine the kinetic effect of HIMP and M-MeI on the growth of LNCaP C-81 cells, cells were seeded into six-well culture plates at a density of 2 x 10^3 cells/cm^2 and maintained in regular culture conditions for 3 days. Cells were then changed to SR medium and maintained for 2 days. One plate of attached cells was harvested and counted as day 0, and the remaining cells were changed to their respective treatment medium: Control (DMSO), HIMP, and M-MeI (10 μM). At each time point, cells were harvested for cell number counting and the remaining cells were fed with fresh media containing respective treatment compound. After cell number counting, cell lysates were prepared for Western blot analysis.

Flow Cytometry Analysis

To determine the compounds' effect on cell cycle, LNCaP C-81 cells were seeded in T25 flasks at a density of 2 x 10^3 cells/cm^2 in regular medium for 3 days, changed to SR medium for 48 hours, and then fed with fresh SR medium containing 10 μM of specified compound. One set of cells was harvested after 3, 5, and 7 days of treatment, respectively, by trypsinization. After cell number counting, an aliquot of cells was pelleted by centrifugation, resuspended in 70% ethanol, and incubated at 4°C for 30 minutes, then washed with PBS and spun down again by centrifugation. The DNA of ethanol-fixed cells was stained using Telford Reagent (PBS, pH 7.4, containing 0.1% Triton X-100, 0.1mM EDTA disodium salt, 0.05mg/ml RNase A (50 U/mg), and 50 mg/ml propidium iodide) at 4°C for 4 hours [29]. Determination of cell cycle

distribution was carried out using a Becton-Dickinson fluorescence-activated cell sorter (FACSCalibur, Becton Dickinson, San Jose, CA, USA) at the UNMC Flow Cytometry Core Facility.

Cell Adhesion Assay

To determine the effect of imidazopyridine derivatives on PCa cell adhesion to plastic-ware surfaces, LNCaP C-81 cells were suspended in 5% FBS 1640 RPMI medium containing 10 μM of respective compounds and incubated for 30 minutes. Cells were then plated in 6-well plates in triplicates at a density of $3x10^3$ cells/cm^2 in respective treatment medium and incubated for an additional hour. Non-attached cells were carefully washed away and the remaining attached cells were stained with a 0.2% crystal violet solution containing 50% methanol. The total number of cells in five fields at 40x magnification per well were counted.

Clonogenic and Soft Agar Colony Formation Assays

The clonogenic cell growth assay on the surface of plastic-wares was conducted as described previously [23,26]. Briefly, LNCaP C-81 cells were plated into 6-well plates under regular culture conditions at three densities: 20, 200, and 2,000 cells per well. Cells were incubated overnight, after which unattached cells were removed and those attached cells were fed with fresh regular medium containing 10 μM of treatment compound. Cells were grown for 9 days with a change of fresh medium every three days. On the 10^{th} day, the medium was removed and cells were washed with ice-cold HEPES-buffered saline, then attached cells were stained with a 0.2% crystal violet solution containing 50% methanol. The experiment was carried out in duplicate.

The effect of imidazopyridine derivatives on anchorage-independent growth of LNCaP C-81 cells was assessed by soft agar assay. Briefly, $5x10^4$ cells were seeded into a 0.25% agarose top layer with a base layer containing 0.3% agarose in 6-well plates. The day after seeding, cell clusters containing more than one cell were excluded from the study. Cells were then fed with 0.5 mL of fresh regular medium containing the respective compound every 3 days for 4 weeks. After the experimental period, colonies were stained with a 0.2% crystal violet solution containing 50% methanol and counted.

Cell Migration Assay

To determine the effect of imidazopyridine derivatives on PCa cell mobility, LNCaP C-81 cell migration was assessed via Boyden chamber assay. Cells were plated at a density of 5×10^4 cells into the upper chamber of 24-well plate transwell inserts. Medium containing 10 μM of treatment compound (solvent alone for control) was placed in both upper and lower chambers of the transwells. Cells were then incubated for 24 hours, after which they were stained with 0.2% crystal violet solution in 50% methanol, and cells remaining in the upper chamber were removed via cotton swab. Cells which had migrated through to the lower chamber were counted at 40x magnification under a microscope.

Immunoblot Analysis

All cells were rinsed with ice-cold HEPES-buffered saline, pH 7.0, harvested via scraping, and lysed in ice-cold lysis buffer containing protease and phosphatase inhibitors. Total cellular lysates were prepared as previously described [19,30]. The protein concentration of the supernatant was determined using a Bio-Rad Bradford protein-assay. For immunoblotting, an aliquot of total cell lysate was electrophoresed on SDS-polyacrylamide gels (7.5%-12%). After being transferred to nitrocellulose membrane, membranes were blocked with 5% non-fat milk

in Tris-buffered saline (TBS) containing 0.1% Tween-20 for 30 minutes at room temperature. Membranes were incubated with the corresponding primary Ab overnight at 4°C. Membranes were then rinsed and incubated with the appropriate secondary Ab for 60 minutes at room temperature. Proteins of interest were detected by an enhanced chemiluminescence (ECL) reagent kit and β-actin was used as a loading control.

Statistical Analysis

Each set of experiments was conducted in duplicate or triplicate as specified in the figure legends, and experiments were repeated independently at least two or three times. The mean and standard error values of all results were calculated and two-tailed student-t test was used to determine significance of results. $p<0.05$ was considered statistically significant.

Results

Dose-Dependent Effect of Imidazopyridine Derivatives on CR PCa cell Proliferation

LNCaP C-81 cells exhibit many biochemical properties as seen in clinical CR PCa, including functional AR expression, AI PSA secretion, and proliferation with intracrine growth regulation [19,21–23] and thus were used as the primary cell model system for testing imidazopyridine compounds. Initially, the dose-dependent effects of HIMP, M-MeI, OMP, and EtOP on LNCaP C-81 cells were tested under regular culture conditions. Cells were treated with 0–10 μM of each compound for 72 hours and cell growth was analyzed via Trypan Blue exclusion assay. Under regular culture conditions, dose-dependent inhibition of cell proliferation was observed for all compounds with estimated IC_{50} values of 6.1 μM (M-MeI), 6.6 μM (EtOP), 7.3 μM (OMP), and 9.3 μM (HIMP) (Fig 2A).

We then examined the effect of the compounds in SR conditions, mimicking ADT conditions. The compounds inhibited cell growth following the dosage response with estimated IC_{50} values of 10.2 μM (M-MeI), 10.5 μM (OMP), 11.6 μM (HIMP), and 16.0 μM (EtOP) (Fig 2A). Interestingly, M-MeI had the greatest inhibitory activity under both growth conditions. Though EtOP had comparable inhibition to M-MeI in regular conditions, it had the least effect under SR conditions. HIMP and OMP were also shown to be less effective than M-MeI under both treatment conditions.

Selective Anti-Proliferative Effect of Compounds on PCa vs. Immortalized Normal Prostate Epithelial Cells

The suppressive effect of each inhibitor on proliferation was investigated using a panel of cancerous and benign prostate epithelial cell lines. AI PCa cells including AR-positive LNCaP C-81 and MDA PCa2b-AI as well as AR-negative PC-3 cells were chosen as representatives of advanced CR PCa. RWPE1 cells, an immortalized benign prostate epithelial cell line, were used to determine the compounds' selectivity. After three days of 10 μM treatment under SR conditions, HIMP, M-MeI, and EtOP all displayed selective inhibition of proliferation of cancerous cells with significantly less effect on non-cancerous RWPE1 cells (Fig 2B). Though OMP was effective against C-81 and PC-3 cells, it was comparatively potent against RWPE1 cells. Overall, the results show HIMP and M-MeI were the most selective, inhibiting PCa cell growth significantly more than RWPE1 cells with M-MeI displaying greater inhibition in all cell lines analyzed.

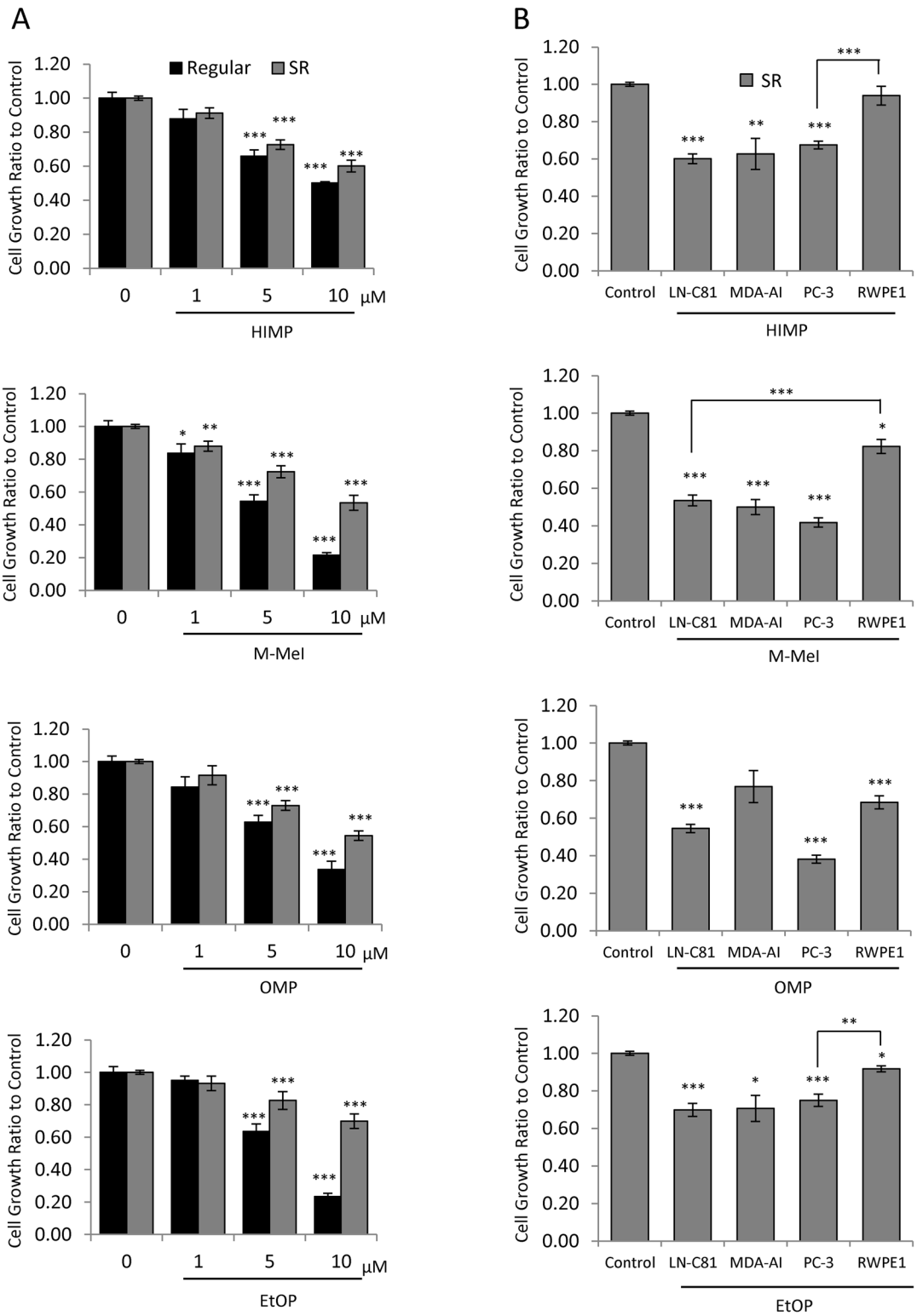


Fig 2. Effects of imidazopyridine derivatives on the proliferation of prostate epithelial cells. (A) Dosage effect of Imidazopyridine derivatives on LNCaP C-81 cells. Cells were plated in six-well plates at 2 x 10^3 cells/cm^2 in regular medium and grown for 72 hours. One set of cells was then fed with fresh regular medium containing 0,1,5, or 10 μM imidazopyridine derivatives with solvent alone for control and grown for an additional 72 hours. Another set of cells was first steroid starved in SR medium for 48 hours then treated with respective compounds in fresh SR media containing 1 nM DHT for 72 hours. All cells were trypsinized and live cell numbers were counted. The experiment was conducted in duplicate wells with 3 sets of independent experiments. The results

presented are mean ± SE; n = 2x3. *p<0.05 **p<0.005 ***p<0.0005. **(B)** Effects of imidazopyridine derivatives on the growth of various PCa cells and immortalized prostate epithelial cells. All cells were plated in six-well plates at the noted density in their respective medium for three days, steroid-starved for two days, then fed with fresh SR medium with 1 nM DHT containing 10 μM imidazopyridine derivatives and grown for three additional days. Cells were trypsinized and live cell number was counted. LNCaP C-81–2 x 10^3 cells/cm^2, MDA PCab2b AI—3 x 10^3 cells/cm^2, PC-3–2 x 10^3 cells/cm^2, RWPEI– 7.5 x10^3 cells/cm^2. All experiments were performed in triplicate wells with 3 sets of independent experiments. Results presented are mean ± SE; n = 3x3. *p<0.05; **p<0.001; ***p<0.0001.

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Suppression of PCa Tumorigenicity by Imidazopyridine Derivatives

The compounds' ability to suppress colony formation in LNCaP C-81 cells was initially accessed by in vitro clonogenic assays for anchorage-dependent cell growth. LNCaP C-81 cells were seeded with 20, 200, and 2,000 cells per well in 6-well plates, and then treated with 10μM of each compound. Upon 10-days of treatment, all compounds significantly inhibited clonogenic growth at 2,000 cells per well as shown in Fig 3A for 2,000 cells per well. While M-MeI and EtOP strongly inhibited colony growth, HIMP and OMP were comparatively less potent. Minimal colony formation was observed at densities of 20 and 200 cells per well.

The soft agar colony formation assay was then performed to determine the compounds' effect on anchorage-independent growth in a 3-dimentional environment. As shown in Fig 3B, cells cultured at a density of 5,000 cells per 35 mm dish for four weeks produced far fewer colonies with smaller colony size when treated with the imidazopyridine derivatives. Compared to control cells treated with solvent alone, M-MeI suppressed colony growth to the greatest extent, reducing the number of colonies by 90 percent with barely visible colony size (Fig 3B). In comparison, EtOP and OMP reduced colony growth with 80 and 70 percent inhibition, respectively, and HIMP had the least effect at about 50 percent inhibition.

To clarify whether these compounds' effect on colony formation is in part due to the inhibition of cell adhesion, the capacity of HIMP, M-MeI, OMP, and EtOP to influence PCa cell adhesion onto the plastic surface of 6-well plates was then investigated. While these compounds had varying degrees of suppression on the ability of LNCaP C-81 cells to adhere at a density of 50,000 cells per well, a similar inhibitory trend was observed to that of clonogenic and soft agar assays (Fig 3C vs. 3A & 3B). M-MeI had the greatest effect and was able to reduce cell attachment by about 40 percent. While HIMP and EtOP were also able to significantly inhibit cell adherence, they did so to a lesser extent; OMP was found to have a minimal effect on the C-81 cells' ability to attach to the plastic surface. Hence, though all compounds belong to the same class of molecules, they influence PCa cell colony formation and adhesion differently.

To investigate the inhibitory ability of these compounds on tumor metastasis, their activity on cell migration was analyzed by transwell migration assay. Interestingly, these compounds were found to have varying degrees of suppression on PCa cell migration. Fig 3D showed that both M-MeI and EtOP were able to significantly reduce LNCaP C-81 cell migration via Boyden Chamber assay over a period of 24 hours by about 30 percent. Comparatively, HIMP and OMP failed to significantly reduce cell migration. Overall, M-MeI was found to exhibit the most potent inhibitory activity on CR PCa cell tumorgenicity.

Effect of Imidazopyridine Compounds on Proliferative and Apoptotic Signaling in CR PCa Cells

It is well established that the majority of CR PCa cells express functional AR which is still required for their growth and survival [22,31,32]. To determine how the compounds suppress PCa cell proliferation, we analyzed their effects on proliferative and apoptotic signaling in AR-positive LNCaP C-81 and MDA PCa2b-AI cells under SR conditions. Fig 4A and 4B showed

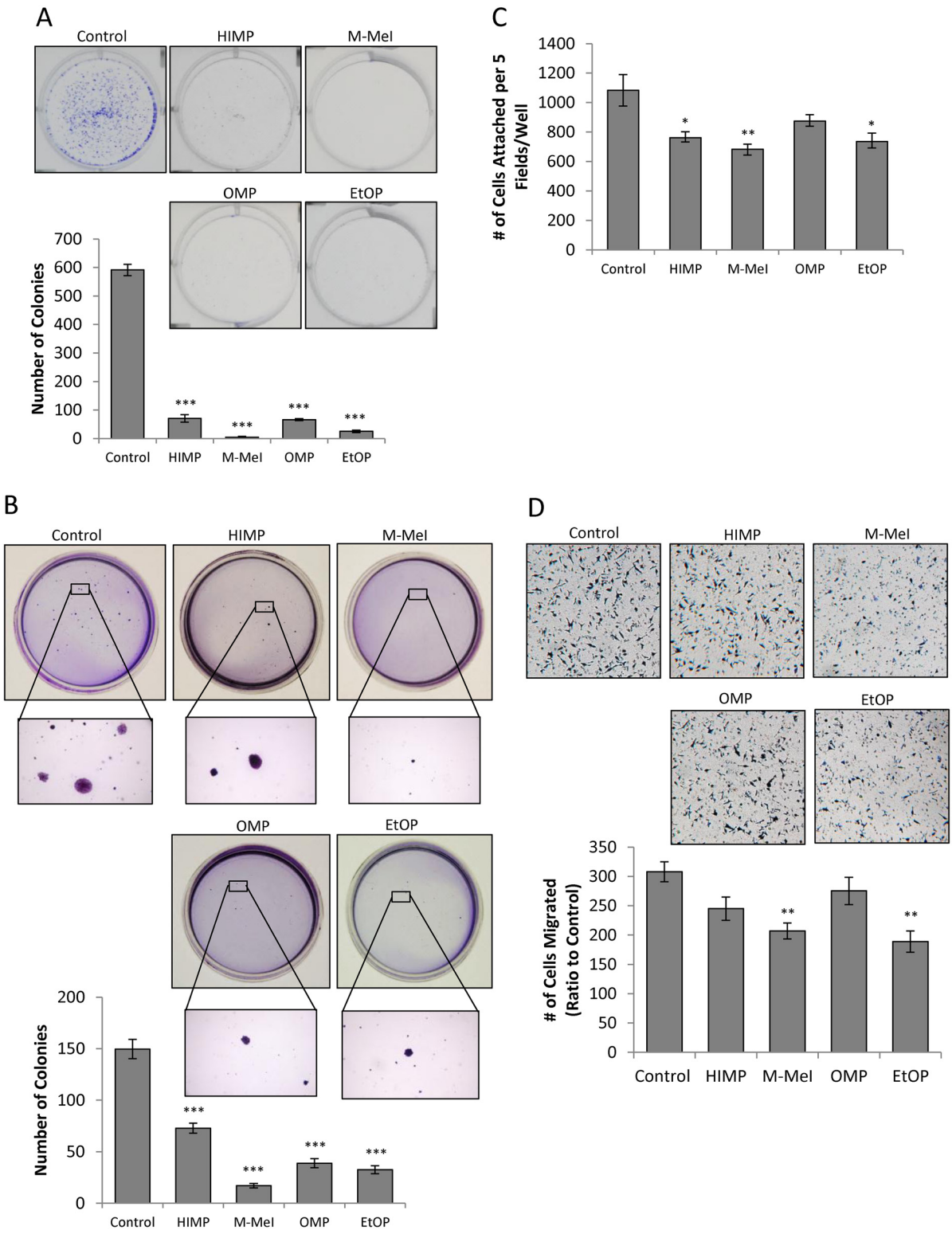

Fig 3. Effects of imidazopyridine derivatives on the tumorigenicity of LNCaP C-81 cells. (A) Clonogenic assay on plastic wares. LNCaP C-81 cells were plated in six-well plates at densities of 20, 200, and 2,000 cells/well. After 24 hours, attached cells were treated with respective compounds at 10 μM concentrations of imidazopyridine derivatives or solvent alone as control. Cells were fed on days 3, 6, and 9 with fresh culture media containing respective inhibitors. On day 10, cells were stained and the number of colonies counted. The photos of representative colony plates were taken from plates seeded with 2,000cells/well, and the number of colonies shown was counted also from plates seeded with 2,000cells/well. Minimal colony formation was observed at

densities of 20 and 200 cells/well. Results presented are mean ± SE; n = 2x3. ***p<0.0001. **(B)** Anchorage-independent soft agar assay. LNCaP C-81 cells were plated at a density of 5 x 10^4 cells/35mm dish in 0.25% soft agar plates. The following day, cells in doublets or greater were marked and excluded from the study. Media were added every three days, and at the end of 5 weeks, colonies formed were stained and counted. Representative images of colonies are shown (above) and the colony number was counted (below). The experiments were performed in duplicate with 3 sets of independent experiments. Results presented are mean ± SE; n = 2x3. *** p<0.0001. **(C)**. Cell adhesion assay on plastic wares. Cells were suspended in treatment media for 30 minutes before being plated in 6-well plates at 3 x10^3 cells/cm^2 using the same treatment media. Cells were allowed to adhere for one hour, fixed and stained by 0.2% crystal violet solution (50:50, water:MeOH). The total number of cells in five fields at 40x magnification for each well was counted. The experiments were performed in triplicate with 3 sets of independent experiments. Results presented are mean ± SE; n = 3x3. *p<0.05; **p<0.01. **(D)**. Cell migration transwell assay. Cell migration was assessed via Boyden chamber. An aliquot of 5 x 10^4 C-81 cells was seeded in the insert of 24-well plates in media containing 10 μM respective compounds with solvent alone for control in both upper and lower chambers. After 24-hour incubation, the migrated cells were stained and those cells remaining in the upper chamber were removed via cotton swab. Cells which had migrated through to the lower chamber were counted. Representative images are shown at 40x magnification. The experiments were performed in triplicate with 3 sets of independent experiments, and the results presented are mean ± SE; n = 3x3, *p<0.05; **p<0.005.

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that, upon 3-day treatments, 10 μM of each compound significantly suppressed LNCaP C-81 and MDA PCa2b-AI cell proliferation.

In LNCaP C-81 cells under SR conditions, though imidazopyridines are known for Akt inhibition, only HIMP and M-MeI inhibited Akt activation as shown by decreased Ser473 phosphorylation (Fig 4C) [15]. Additionally, while all compounds reduced AR protein levels in LNCaP C-81 cells; M-MeI and EtOP were more potent. Importantly, a similar trend was observed in AR-regulated pro-proliferative proteins. M-MeI and EtOP reduced levels of p66Shc, a 66kDa Src-homologous collagen homologue, cyclin D_1, and PCNA, while HIMP and OMP had minimal effects [33–36]. We also analyzed Stat5 phosphorylation at Y694, which aids in the translocation of AR to the nucleus and is a regulator of cylcin D_1 synthesis [37,38]. Unexpectedly, M-MeI slightly increased Stat5 activation in C-81 cells while EtOP suppressed activation; HIMP and OMP had no effect. Though all compounds diminished anti-apoptotic Survivin protein, their treatment elevated Bcl_{XL}, another anti-apoptotic protein [39,40]. The compounds' effect on p53, a regulator of cell survival and inducer of apoptosis, varied with M-MeI slightly lowering p53 levels, and EtOP slightly increasing them; HIMP and OMP had no significant effect [41].

As shown in Fig 4D, in MDA PCa2b-AI cells under SR conditions, AR inhibition was similar to that of LNCaP C-81 cells: M-MeI and EtOP greatly suppressed AR levels while HIMP and OMP had minimal effects. This also correlated with lower levels of AR-regulated p66Shc, cyclin D_1, and PCNA in M-MeI-treated cells, all of which promote cell growth. As seen in C-81 cells, in MDA PCa2b-AI cells HIMP and OMP had no inhibitory effect on Stat5 activation, while EtOP suppressed Y695 phosphorylation. M-MeI, however, strongly reduced Stat5 phosphorylation in MDA PCa2b-AI cells where it had increased it in C-81 cells. Also, while the compounds' effect on p53 remained similar in MDA-AI cells compared to C-81 cells, their effects on anti-apoptotic Survivin and Bcl_{XL} proteins were altered. In MDA-AI cells, HIMP, OMP and EtOP increased levels of Survivin while Bcl_{XL} remained unchanged relative to control cells suggesting these proteins are not essential to growth inhibition. Because Akt phosphorylation at Ser473 in MDA PCa2b-AI cells was undetectable under SR conditions, these cells were instead treated in regular culture medium for three days and all compounds were found to reduce Akt activation (Fig 4E).

In summary, of the four compounds tested, M-MeI was the most potent inhibitor of proliferation in both LNCaP C-81 and MDA PCa2b-AI cell lines under SR conditions. It also consistently reduced AR and AR-regulated proteins as well as Akt Ser473 phosphorylation in both cell lines, suggesting these pathways are involved in imidazopyridine inhibition of CR PCa cell growth.

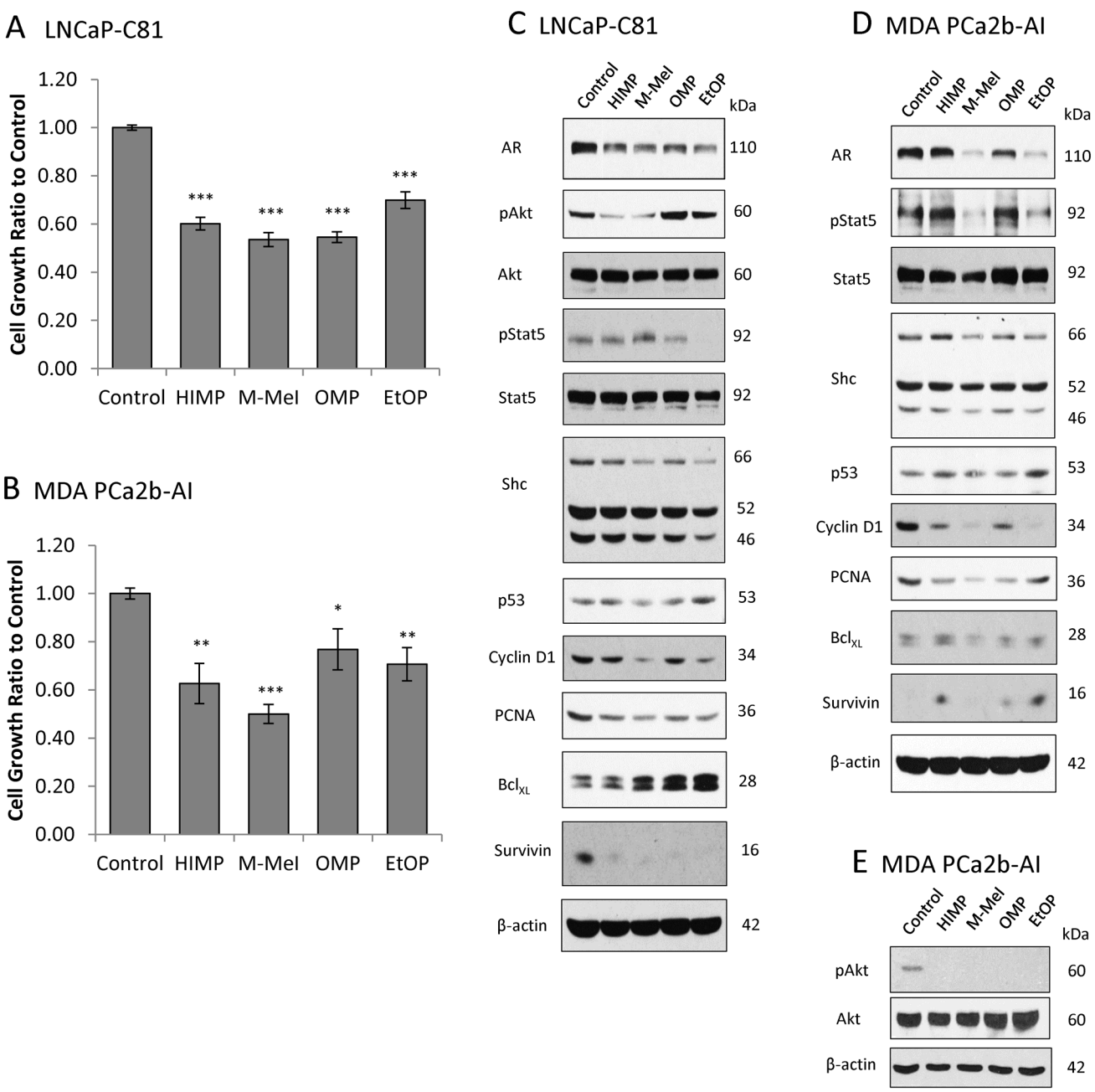

Fig 4. Effects of imidazopyridine derivatives on PCa proliferative and apoptotic signaling under SR conditions. (A) LNCaP C-81 cells were plated in triplicate in T25 flasks at 4 x 10^3 cells/cm^2 in regular medium, grown for 72 hours then steroid starved for 48 hours. Cells were then treated with 10 μM imidazopyridine derivatives or DMSO as control for an additional 72 hours under SR conditions. Cells were trypsinized and live cell numbers counted via Trypan Blue assay. The experiments were performed in triplicate with 3 sets of independent experiments. *** p<0.0005. **(B)** MDA PCa2b-AI cells were grown, treated, and counted under conditions as described above in (A) for LNCaP C-81 cells. Results presented are mean ± SE; n = 3x3. *p<0.05; **p<0.005; ***p<0.0005. **(C)** LNCaP C-81 total cell lysate proteins were collected from (A) after cell number counting. Those cells were grown in SR conditions and analyzed for phosphorylated Akt and STAT5, as well as total AR, Akt, Shc, p53, cyclin B_1, cyclin D_1, PCNA, Bcl_{XL}, and Survivin protein levels. β-actin protein level was used as a loading control. Similar results were observed in two sets of independent experiments. **(D)** MDA PCa2b-AI total cell lysate proteins from (B) after cell number counting. Cells were grown in SR conditions and analyzed for phosphorylated STAT5, as well as total AR, Shc, p53, cyclin B_1, cyclin D_1, PCNA, Bcl_{XL}, and Survivin protein levels. β-actin protein level was used as a loading control. Similar results were observed in three sets of independent experiments. **(E)** MDA PCa2b-AI total cell lysate proteins from cells grown in regular conditions were analyzed for phosphorylated Akt and total Akt. β-actin protein level was used as a loading control. Similar results were observed in three sets of independent experiments.

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Kinetic Effect of HIMP and M-MeI on LNCaP C-81 Cells under SR Conditions

Since HIMP and M-MeI exhibited the most selectively potent activity, these two compounds were investigated further by kinetic analysis in LNCaP C-81 cells under SR conditions for clinical relevance. As shown in Fig 5A, both HIMP and M-MeI began to show significant inhibition of cell proliferation on day three of treatment, and this trend continued through day seven. It should also be noted M-MeI exhibited greater growth suppression than HIMP at every time point analyzed.

Cell lysates were collected from each time point and Western Blot analysis was performed (Fig 5B). Our results showed M-MeI-treated cells had decreased levels of AR and PSA, an androgen-regulated protein, as well as cell cycle proteins cyclin B_1 and PCNA as seen at 5-day treatment. Meanwhile, HIMP treatment decreased PSA protein level upon 7-day treatment but not AR protein level. A similar trend was observed in pro-proliferation proteins as HIMP had no effect on cyclin B_1 and marginal effect on PCNA. It should be noted, as the level of active unphosphorylated cyclin B_1 decreased (lower band), the inactive phosphorylated cyclin B_1 protein level increased (upper band) [42]. The data may indicate arrestment of the cell cycle.

We also investigated the effects of HIMP and M-MeI on pro-apoptotic proteins Bax as well as p53 vs. anti-apoptotic Bcl-X_L [40]. Treatment with both compounds dramatically increased Bax and p53 protein level by day seven and correlated with the observed decrease in cell proliferation. Interestingly, both compounds initially increased Bcl-X_L levels on days one and three, and had no effect on Bcl-X_L as treatment continued (Fig 4B). Thus both compounds seem to induce apoptosis by increasing levels of pro-apoptotic proteins instead of inhibiting anti-apoptotic proteins, while M-MeI also reduces pro-proliferative proteins as well as AR signaling.

To further investigate the kinetic effect of HIMP and M-MeI, cell cycle analysis was performed via flow cytometry. The cell cycle analyses (Fig 5C) revealed that upon 7-day treatment, M-MeI reduced LNCaP cell proliferation by 50% as indicated by the decreased percentage of cell population in S phase. M-MeI also increased the percentage of cells undergoing apoptosis nearly four-fold at day seven relative to control cells. At the same time, HIMP had no significant effect on percentage of cells in S phase and increased the percentage undergoing apoptosis two-fold by day seven. Interestingly, the population of cells treated with M-MeI, but not HIMP, accumulated in G2 phase following the time course of treatment, i.e., only a marginal increase on day 3 and about a 50% increase in day 5 (S1 Fig). On day-7, the percentage of cells in G2 phase doubled to about 49% in M-MeI treated cells compared to 24% in control cells (Fig 5C). In parallel, on day 7, the HIMP-treated cells were only slightly increased from about 24% to 27% in G2-phase. These results clearly indicate M-MeI blocks cell cycle at G2 phase.

Discussion

Given the poor 5-year 29% survival rate of metastatic CR PCa patients, there is a clear need for advancement in treatment alternatives [14]. First line treatment of PCa usually involves ADT by means of surgical castration or chemical castration, such as luteinizing hormone-releasing hormone treatment, coupled with anti-androgens. Treatment with classic anti-androgens such as flutamide or bicalutamide (Casodex), which competitively inhibit androgen binding to AR, can be effective for 1–3 years before patients subsequently become unresponsive and eventually relapse. Relapse can occur for a number of reasons such as deregulation of AR cofactors, AR overexpression, splicing mutations resulting in constitutively active AR, or mutations allowing AR activation by competitive inhibitors [43]. In the case of the latter, upon discontinuation of flutamide or bicalutamide treatment, patients often experience a period of anti-androgen withdrawal syndrome (AAWS) characterized by a decline in serum PSA levels and tumor

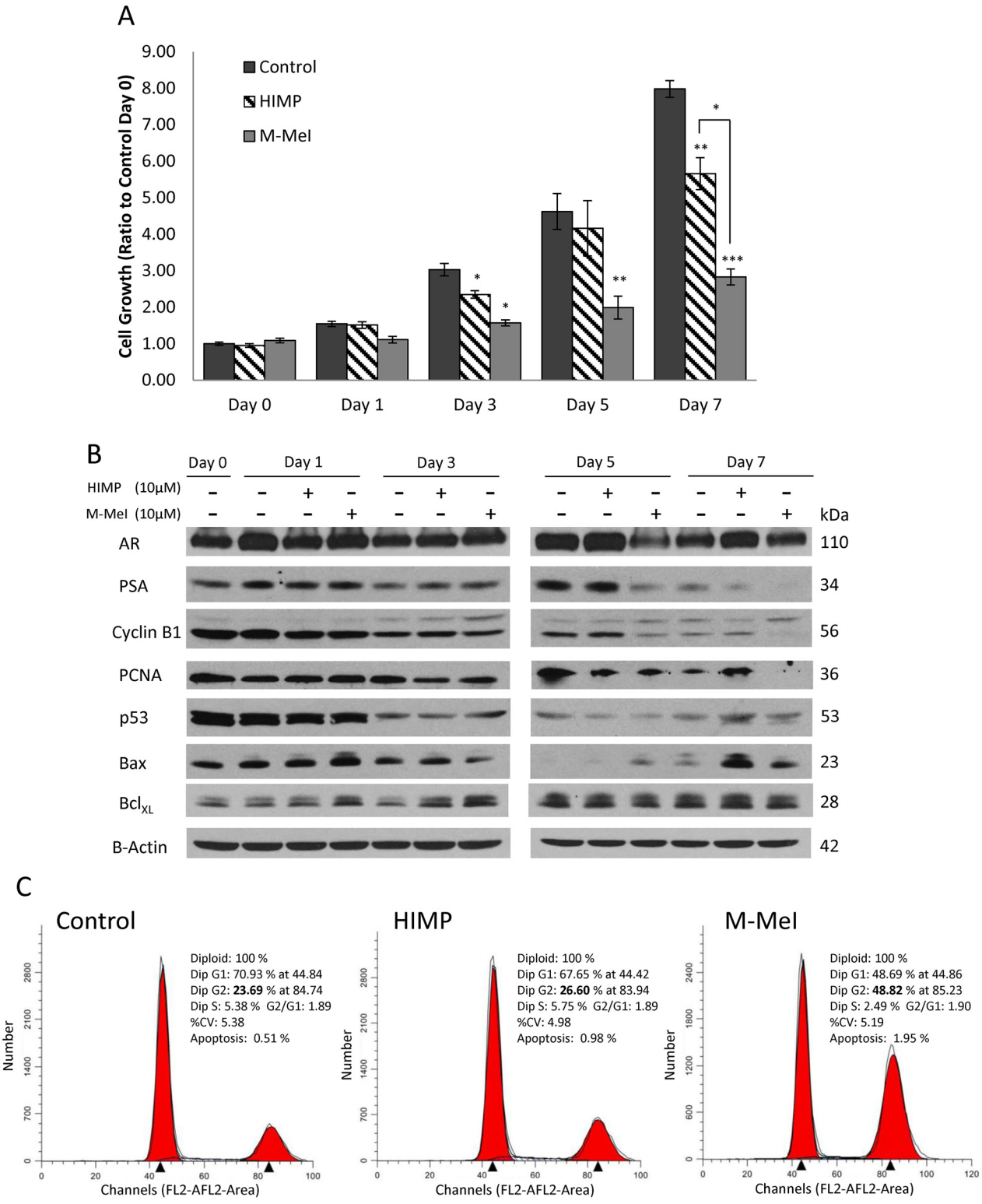

Fig 5. Kinetic analysis of HIMP and M-MeI's effects on LNCaP C-81 cells under steroid-deprived conditions. (A) Cells were plated in six-well plates at 2 x 10^3 cells/cm^2 in regular medium for three days, then steroid-starved for 48 hours followed by treatment with 10 μM HIMP or M-MeI in SR medium containing 1 nM DHT. Solvent alone was used for controls. On day 0, 1, 3, 5 and 7, one set of cells in duplicates from each group was harvested for live cell counting. Remaining cells were replenished with fresh respective medium. The experiments were performed in duplicates with 3 sets of independent experiments. Results presented are mean ± SE; n = 2x3. *p<0.05; **p<0.005; ***p<0.0005. **(B)** Total cell lysate proteins from HIMP- and M-MeI-treated C-

81 cells from (A) were collected and analyzed for AR, cPSA, cyclin B_1, PCNA, Bax, p53, and Bcl-X_L proteins. β-actin protein level was used as a loading control. **(C)** Histograms of cell cycle distributions of LNCaP C-81 cells upon 7 days of HIMP and M-MeI treatments. Cells were plated in T25 flasks at 2 x 10^3 cells/cm^2 in regular medium for three days, then steroid-starved for 48 hours followed by treatment with 10 μM HIMP or M-MeI in SR medium with 1 nM DHT and solvent DMSO alone as control. One set of cells from each group was harvested after 3, 5, and 7 days treatment for flow cytometric analysis. Similar results were obtained from two sets of independent experiments. The data shown were representative results of 7-day treatments.

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regression [43,44]. In some cases, these patients will respond to treatment with alternative anti-androgens, however, patients will again eventually become unresponsive to treatment and develop advanced PCa, commonly referred to as "castration-resistant" (CR) [45]. While more effective anti-androgens such as enzalutamide, which possesses a 5-fold higher binding affinity to AR compared to bicalutamide, are now available, mutations allowing AR activation by enzalutamide have been reported and tumors continue to become CR over time [46]. The therapeutic effect of second generation anti-androgens such as enzalutamide and abiraterone acetate implies most cancer cells, which still express functional AR, require AR signaling to evade traditional regulatory mechanisms to survive androgen deprivation strategies [7,8,47]. Despite the effectiveness of ADT and anti-androgen treatment strategies to delay the progression of PCa, many patients still develop the CR phenotype and thus there is an urgent need for alternative therapeutic targets to AR. Other signaling pathways, such as the PI3K/Akt-mediated cell survival pathway, may act to supplement the lack of AR stimulation under androgen-deprived conditions, bypassing the effect of ADT. Therefore, in this study we investigate novel therapeutic agents which are capable of targeting multiple biochemical pathways critical to CR PCa cell growth and progression.

In this study, four novel imidazopyridine derivatives were investigated to determine their viability as therapeutic agents for CR PCa. LNCaP C-81 cells were chosen as our primary experimental cell model because they possess many biochemical properties common to CR PCa, including expression of functional AR, AI PSA secretion and proliferation, and expression of enzymes required for the synthesis of androgens from cholesterol [19,21,22,31,47]. We initially showed that all four compounds are effective inhibitors of CR PCa cell growth in the LNCaP C-81 cell line model, though to varying degrees with M-MeI as the most potent under both regular and SR conditions (Fig 2A). Additionally, the effects of the imidazopyridine derivatives on cell proliferation differed between compounds as well as cell lines. Of those compounds investigated, HIMP and M-MeI displayed broad-spectrum growth inhibition of multiple CR PCa cell lines, including both AR-positive and AR-negative cells. Most importantly, these compounds exhibited more potent inhibition of proliferation in PCa cells than benign RWPE1 cells (Fig 2B).

We further investigated the effects of each compound on various biological activities critical to malignant tumor progression. Colony formation, cell adhesion, and migration are vital malignant processes exhibited by many cancer cells. In both anchorage-dependent and anchorage-independent growth assays, M-MeI and EtOP dramatically reduced the number of colonies formed as well as colony size. This may partially be due to M-MeI and EtOP's ability to inhibit cell adhesion. To investigate the effect of these compounds on cell migration, the transwell migration assay was used to analyze LNCaP C-81 cell motility. Again, M-MeI and EtOP were found to be potent inhibitors of C-81 cell migration while HIMP and OMP had no significant effect. Interestingly, this correlates with M-MeI and EtOP's ability to reduce AR protein level as shown in Fig 4C where HIMP and OMP fail to influence AR. This suggests AR inhibition may be crucial for the inhibition of these malignant processes. However, while EtOP was an effective inhibitor of PCa tumorgenicity, it was not as effective at growth suppression as M-MeI. This may be due to EtOP's failure to prevent Akt activation whereas M-MeI successfully inhibits Akt phosphorylation at S473 (Fig 4C).

The PI3K/Akt signaling pathway is proposed to be pivotal to the growth and survival of CR PCa cells; dysregulation of this pathway is shown to contribute to resistance to treatment [31,48–54]. In clinical studies, for example, treatment with AR inhibitor bicalutamide is shown to progressively increase Akt signaling in patients in correlation with their Gleason grades [51]. In parallel, PCa treated with chemotherapeutic agent docetaxel possess increased Akt activation in patient tumors [52]. Other *in vitro* studies similarly demonstrated LNCaP cells grown in SR medium for extended periods of time have increased Akt activation which may compensate for a lack of androgen signaling [12]. Indeed, there is high prevalence of PI3K/Akt/mTOR pathway activation in CR PCa and emerging studies show inhibitors targeting the PI3K/Akt pathway are rapidly entering into clinical trials [14,53–55]. Therefore, the PI3K/Akt signaling axis is a promising next-in-line therapeutic target and its inhibition in conjunction with ADT and anti-androgens may improve patient survival.

Initially, imidazopyridines have been shown to possess Akt kinase inhibitory activity and are effective suppressors of tumor growth and advancement in a number of carcinomas, including PCa [15,16,18]. Interestingly, while all four derivative compounds inhibited Akt phosphorylation at Ser473 in MDA PCa2b cells; only HIMP and M-MeI inhibited its phosphorylation in LNCaP C-81 cells. It should be noted because Akt signaling in MDA PCa2b-AI cells was too weak to detect under SR conditions, it was observed under regular conditions. It is thus possible the differential inhibition of Akt activation in MDA-AI vs. LNCaP C-81 cells was in part due to the difference between regular and SR growth conditions (Fig 4C–4E).

While HIMP is a strong inhibitor of Akt activation in both cells, it fails to consistently down-regulate AR, a long established target of PCa therapy. Supportively, western blot analysis showed HIMP inconsistently affects AR protein levels (Figs 4D and 5B), a phenomena which has previously been observed when CR PCa cells are treated with PI3K/Akt inhibitors [12,18]. Furthermore, HIMP had little shift in potency between regular conditions and SR conditions (Fig 2A), suggesting HIMP's mechanism of inhibition is relatively androgen-independent. In addition, HIMP is a potent inhibitor of Akt phosphorylation at Ser473 and also acts to induce pro-apoptotic p53 and Bax proteins (Figs 4C, 4D and 5B) [27]. Together, these results suggest HIMP inhibits CR PCa growth by suppressing cell survivability but not androgen signaling.

While both HIMP and M-MeI were found to have promising selective growth suppression, M-MeI was shown to have greater efficacy (Figs 2 and 4). M-MeI is a derivative of HIMP, possessing a methyl group on the para position of the benzene ring (Fig 1). This modification apparently allows M-MeI to suppress AR protein level as well as Akt activation; there is also a noticeable change in M-MeI's IC_{50} value between regular and SR conditions, indicating its mechanism of growth inhibition includes the suppression of AR signaling (Fig 4C–4E). Supportively, as shown in Fig 5B, upon M-MeI treatment, there is a consistent, progressive decrease in AR protein. This coincides with a decrease in PSA, a target of AR, as well as p66Shc protein level, a protein regulated by androgens which is involved in cell proliferation and apoptosis (Fig 4C and 4D). Downstream pro-proliferative markers, cyclin D_1 and PCNA were also down-regulated over-time to a greater extent by M-MeI compared to HIMP. In addition, STAT5 signaling is proposed to be involved in the transition from androgen-sensitive to CR PCa and activated STAT5 can enhance nuclear translocation of AR [40]. Interestingly, as seen in Fig 4C and 4D, while M-MeI inhibited Stat5 phosphorylation at Y694 in MDA-AI cells, it increased Y694 phosphorylation in LNCaP cells. This inconsistent effect suggests inhibition of STAT5 activation is not vital to M-MeI's suppression of PCa. Furthermore, M-MeI strongly inhibited the phosphorylation of pro-survival protein Akt (Ser473) (Fig 4C–4E) as well as induced pro-apoptotic proteins p53 and Bax (Fig 5B) [27]. Thus, M-MeI's anti-tumorigenic effect on PCa is due to its strong inhibition of both Akt and AR signaling pathways.

Unexpectedly, the kinetic analysis revealed an interesting phenomenon: upon HIMP and M-MeI treatments over time, both compounds, though to a greater extent by M-MeI, decreased the protein level of active unphosphorylated cyclin B_1 (Fig 5B, lower band) and increased the inactive phosphorylated band (upper band) (Fig 5B) [41]. Cyclin B_1, a key regulator of mitosis, is phosphorylated during the G2 phase and becomes unphosphorylated upon the cell reaching M phase [41]. To validate further, we performed cell cycle analysis on HIMP- and M-MeI-treated cells by flow cytometry. Compared to control cells, M-MeI-treated cells had lower percentages of cells in S phase, corresponding with a decrease in cell proliferation, and twice the percentage of cells in the G2 phase. Minimal changes were observed in HIMP-treated cells, although both treatments increased the percentage of cells undergoing apoptosis (Fig 5C). Together, this data indicates these imidazopyridine derivatives function to inhibit the transition of PCa cells between G2 and M phases, though the exact mechanism remains to be elucidated.

In summary, our data shows imidazopyridine derivatives exhibit inhibitory activity of tumorgenicity in CR PCa cells. Each compound displayed differential effects on Akt and AR signaling pathways, and further studies are needed to determine the mechanism by which these molecules suppress the growth of CR PCa. Among the four compounds investigated, M-MeI was found to suppress multiple signaling pathways related to PCa progression, including classical target AR as well as the Akt survival pathway, making it a promising candidate for future therapeutic studies. Importantly, this compound displays selective growth inhibition, having a significantly greater suppressive effect on PCa compared to benign prostate epithelial cells. Thus, M-MeI can serve as a lead compound for imidazopyridine side-chain modifications, which could yield more potent, selective agents to improve the treatment of CR PCa patients. Future investigation to elucidate M-MeI's specific mechanism of inhibition and *in vivo* studies will help better determine its potential as a therapeutic agent.

Supporting Information

S1 Fig. Histograms of cell cycle distributions of LNCaP C-81 cells upon 3 and 5 days of HIMP and M-MeI treatments. Cells were plated in T25 flasks at 2 x 10^3 cells/cm^2 in regular medium for three days, then steroid-starved for 48 hours followed by treatment with 10 μM HIMP or M-MeI in SR medium with 1 nM DHT and solvent DMSO alone as control. One set of cells from each group was harvested after 3, 5, and 7 days treatment for flow cytometric analysis. Similar results were obtained from two sets of independent experiments. The data shown were representative results of 3- and 5-day treatments.
(TIF)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: MFL MAI SM. Performed the experiments: MAI SM TR KH JGD. Analyzed the data: MAI SM MFL. Contributed reagents/materials/analysis tools: XRB ASL ND. Wrote the paper: MAI SM JGD. Reviewed the manuscript: MFL SKB XRB SM.

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